A VALIDATED SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND TELMISARTAN IN PURE, PHARMACEUTICALS AND IN BIOLOGICAL SAMPLE BY UV SPECTROPHOTOMETRY, RP-HPLC AND HPTLC METHOD

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In partial fullfilment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

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Under the Guidance of

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(Accredited by "NAAC" with a CGPA of 2.74 on a four point scale at "B" grade)

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MAY 2012

CERTIFICATE

This is to certify that the research work entitled **A VALIDATED SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND TELMISARTAN IN PURE, PHAMACEUTICALS AND IN BIOLOGICAL SAMPLE BY UV SPECTROPHOTOMETRY, RP-HPLC AND HPTLC METHOD** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fullfilment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **VIJAYASHANTHI. S (REG.NO.26106132)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2011-12.

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Above all I dedicate myself and my work to **Almighty,** who is the source of knowledge and for showering all his blessings and grace upon me.

VIJAYASHANTHI. S

Dedicated

To

My

FAMILY

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INTRODUCTION

1. INTRODUCTION

1.1 INTRODUCTION`

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of elements or compounds contained in them. In analytical chemistry, it is of prime importance to obtain information about the qualitative and quantitative comparison of substance and chemical species.

1.2 UV SPECTROSCOPY

[A.H. Beckett and J.B. Stenlake, 2005, B.K. Sharma, 2000, chatwal, Willard et.al., 1986]

The technique of ultra violet spectrophotometry is one of the most frequently employed methods in Pharmaceutical Analysis. It involves the measurement of the amount of UV (400-200nm) radiation absorbed by a substance in solution. When a beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of the light occur.

This is due to

a) Reflections at the inner and outer surfaces of the cell.

b) Scatter by particles in the solution.

c) Absorption of light by molecules in the solution.

QUANTITATIVE SPECROPHOTOMETRIC METHODS

[A.H.Beckett, and J.B.Stenlake, 2001]

Preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength may quickly carry out the assay of an absorbing substance. The wavelength normally selected is a wavelength of maximum absorption (λ_{max}) where small error in setting the wavelength scale has little effect on the measured absorbance.

Ideally, the concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurement are optimal.

1. **Use of Standard Absorptivity Value:**

   Absorptivity value $A^{1\%}_{1cm}$ or € avoids the need of standard solution of reference substance. It is advantageous where it is difficult or expensive to get pure sample of reference substance.

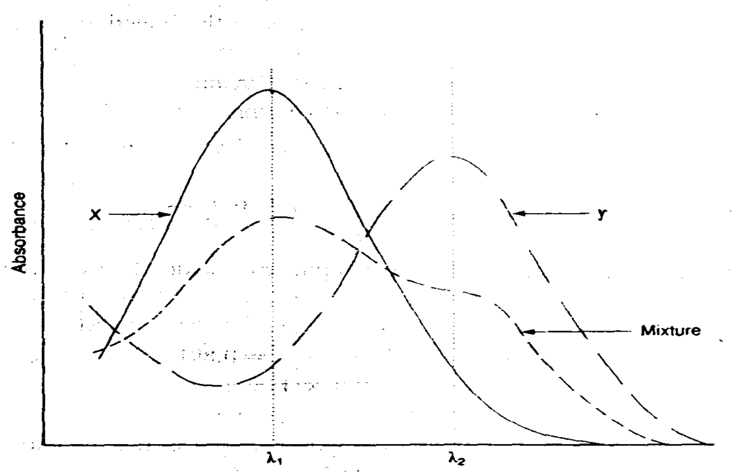
2. **Assay of Substances in Multi Component**

Multi components assays are done by several methods in which concentration of interfering substances should be considered which include.

1. Simultaneous equation method.
2. Absorbance ratio method.
3. Derivative spectroscopic.

SIMULTANEOUS EQUATION METHOD

If a sample contains two, absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other as shown in Fig, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).



The Information required is:

a) The absorptivities of X at λ_1 and λ_2, ax_1 and ax_2, respectively

(b) The absorptivities of Y at λ_1 and λ_2, ay_1 and ay_2, respectively

(c) The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let C_x and C_Y, be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance's of x and y,

At $\lambda 1$ $\quad A_1 = a_{x1}bc_x + a_{y1}bc_y$ ----------- (1)

At λ_2 $\quad A_2 = a_{x2}bc_x + a_{y2}bc_y$ ----------- (2)

For measurement in 1cm cells, b = 1.

Rearrange eq. (2)

$$C_Y = \frac{A_2 - a_{x2}c_x}{a_{y2}}$$

Substituting for C_Y in eq, (1) and rearranging gives

$$C_x = \frac{A_2a_{y1} - A_1a_{y2}}{a_{x2}a_{y1} - a_{x1}a_{y2}} \qquad (3)$$

and

$$C_y = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2} \qquad (4)$$

As an exercise we should derive modified equations containing a symbol (b) for path length, for application in situations where A_1 and A_2 are measured in cells other than 1 cm path length.

$$\frac{A_2/A_1}{Ax2/ax1} \quad \text{and} \quad \frac{ay_2/ay_1}{A_2/A_1}$$

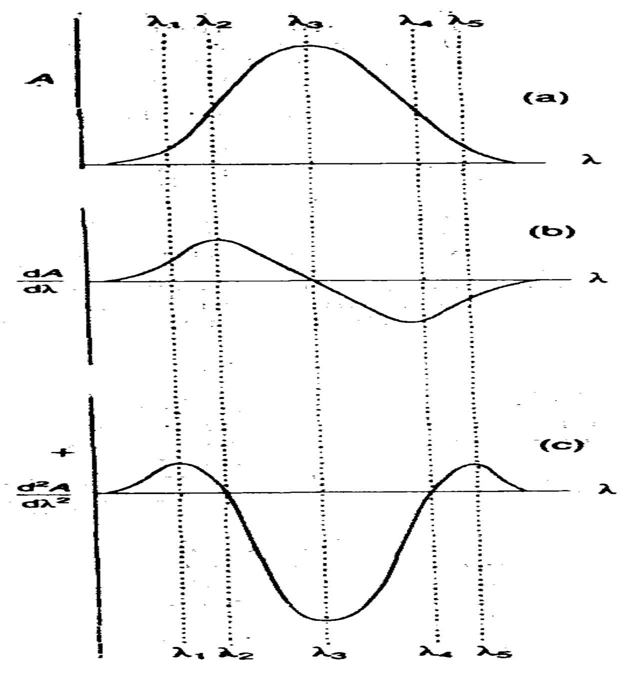
The criteria are that the ratios should lie outside the range 0.1-2.0 for the precise determination of Y and X respectively. An additional criterion is that the two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbance.

DERIVATIVE SPECTROPHOTOMETRY

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D° spectrum.

The first derivative (D^1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ. At λ2 and λ4, the maximum positive and maximum negative slope respectively in the D°. Spectrums correspond with maximum and a minimum respectively in the D^1 spectrum. The λ_{max} at λ_3 is a wavelength of zero slopes and gives dA/d λ 0, i.e. a cross-over point, in the D1 spectrum.

The second derivative (D^2) spectrum is a plot of the curvature of the D° spectrum against wavelength or a plot of $d^2A/d\lambda^2$ Vs λ.



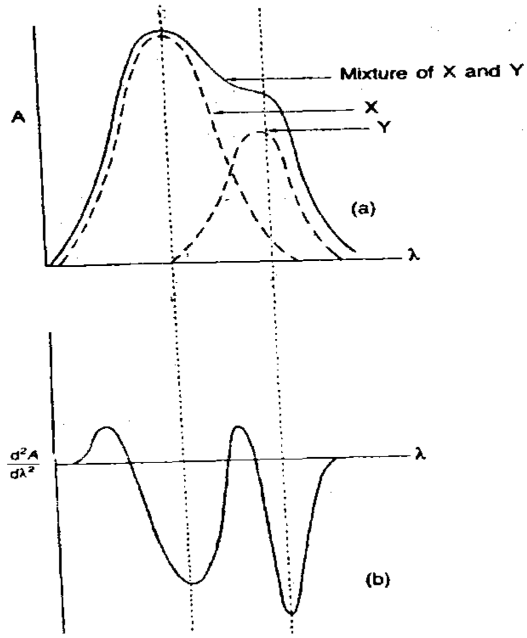
The zeroth (a), first (b) and second (c) derivative spectra of a Gaussian band. Wavelengths of maximum slope and zero curvature in the D° spectrum correspond with cross-over points in the D^2 spectrum.

In summary, the first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ_{max} of the absorption band.

These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. This is because 'the derivative amplitude (D), i.e. the distance from a maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (14') raised to the power (n) of the derivative order.

Thus, $D \alpha (1/W)$

Consequently, substances of narrow spectral bandwidth display larger derivative amplitudes than those of broad bandwidth substance



(a) The individual spectra of two components X and Y in admixture and their combined spectrum (b) The second derivative spectrum of the mixture showing improved resolution of the individual bands.

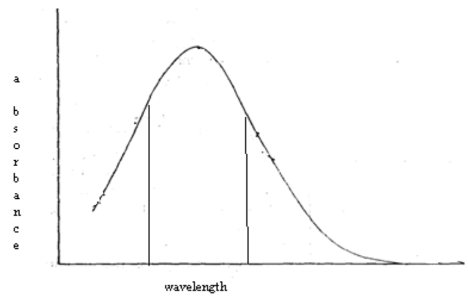
Apart from quantitative analysis, the application of UV is extended to following

1. Structure determination.
2. Quantitative analysis→ it is limited because absorption band tend to be broad.
3. Determination of strength of hydrogen bonding.
4. Study of charge transfer complexes.
5. Study of chemical rings
6. Study of isomerism.

AREA UNDER THE CURVE METHOD (Telekone *et al*., 2010)

The area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength area is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. In combination drugs λ_1 and λ_2 denotes the wavelength ranges of the components. The integrated value of absorbance in the wavelength ranges of both the drugs are substituted in the simultaneous equation to get the concentration of the drugs.

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \text{ And } c_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$



GEOMERTIC CORRECTION METHOD:

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected. Consider an absorption spectrum (Fig. a) comprising the spectrum of analyte (Fig. b) and that of the background absorption (Fig. c).If the wavelengths $\lambda_{1,}$ λ_2 and λ_3 are selected so that the background absorbance's B_1, B_2, and B_3 are linear, then the corrected absorbance, D, of the drug may be calculated from the three absorbance's A_1, A_2, and A_3 of the sample solution at λ_1, λ_2 and λ_3 respectively, as follows.

Let *v*D and *w*D be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively, i.e. *v* and *w* are the absorbance ratios *v*D/D and *w*D/D respectively.

$$\text{Therefore } B_1 = A_1\text{-}vD,\ B_2 = A_2\text{-}D \text{ and } B_3 = A_3\text{-}wD$$

Let y and z be the wavelength intervals ($\lambda_2 - \lambda_1$) and ($\lambda_3 - \lambda_2$) respectively.

Then

$$\frac{B1 - B3}{B2 - B3} = \frac{y + z}{z} \text{ (Similar triangles)}$$

$$\text{Therefore } zB1 = (y + z)\ B_2\text{-}yB_3$$

$$z\ (A_1 - vD) = (y + z)(A_2 - D)\text{-}y(A_3 - v\ D)$$

Rearranging:

$$D = \frac{y(A2 - A3) + z(A2 - A1)}{y(\ 1 - w) + z(1 - v)}$$

Where,

$y = (\lambda_2 - \lambda_1)$

$z = (\lambda_3 - \lambda_2)$

$A_{1=}$ Absorbance of the sample solution at λ_1

$A_{2=}$ Absorbance of the sample solution at λ_2

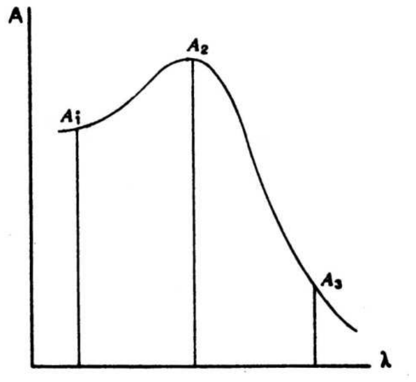
$A_{3=}$ Absorbance of the sample solution at λ_3

$v = v$D/D [absorbance ratio of drug in methanol (without serum) at λ_1 and λ_2]

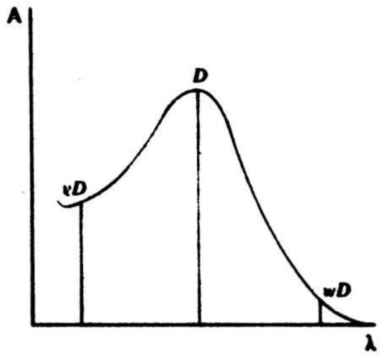
$w = w$D/D [absorbance ratio of drug in methanol (without serum) at λ_3 and λ_2]

This is general equation which may be applied in any situation where A_1, A_2 and A_3 of the sample, the wavelength intervals y and z and the absorbance ratios v and w are known. The values of v and w are determined experimentally using a solution of drug only. The concentration of the drug is calculated from the corrected absorbance D using any of the normal procedures.

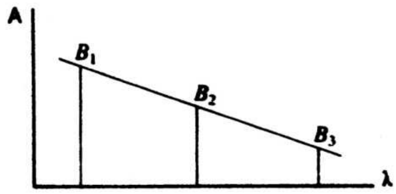
It should be noted that the three-point correction procedures are simply algebraic calculations of what the baseline technique in infrared spectrophotometry does graphically.



(a)



(b)



(c)

CHROMATOGRAPHY [Willard *et al*., P.D Sethi 2001]

Chromatography is differential migration processes where sample components are selectively retained by stationary phase. High Performance Liquid Chromatography is a convenient separation technique used for wide types of samples with exceptional resolving power, speed and nano molecular detection levels. It is an analytical chromatographic technique that is useful for separating ions / molecule that are dissolved in a solvent

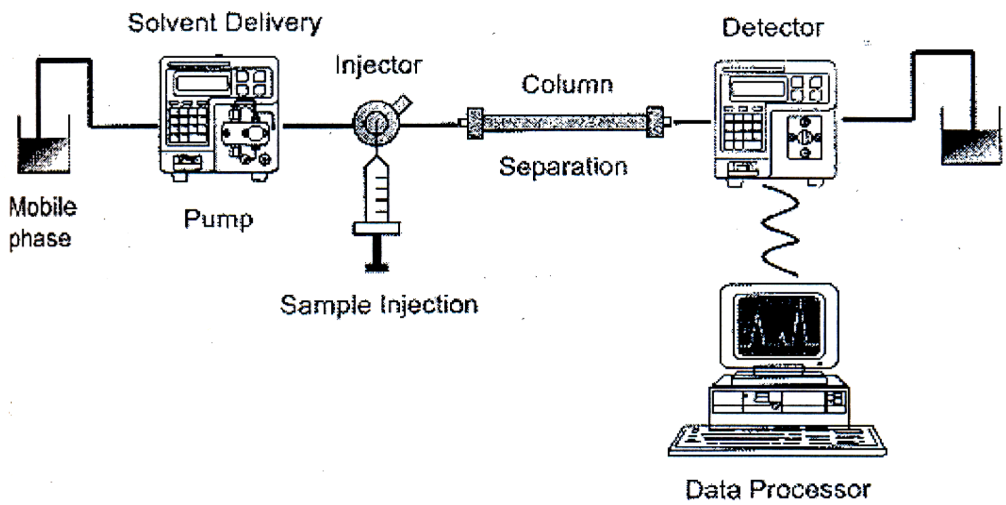
In this two mutually immiscible phases are brought in to contact one phase is stationary and other is mobile. Species in the sample undergo repeated interactions (partitions) between the mobile phase & stationary phase. The components are gradually separated in to bands in mobile phase. It is an analytical chromatographic technique that is useful for separating ions / molecule that are dissolved in a solvent.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

HPLC BASIC INSTRUMENTATION



HIGH PERFORMANCE THINLAYER CHRPMATOGRAPHY: [P.D Sethi 2001]

A method which satisfies the basic requirement for qualitative and quantitative analysis and also capable of being used by personnel with minimum of technical training and under reasonable laboratory facilities, is High Performance Thin Layer Chromatography (HPTLC).

Thin Layer chromatography can be used as a qualitative tool for separation of simple mixtures where low cost and simplicity are required, or else it can be used as a powerful separation tool for quantitative analysis, the later now referred to as HPTLC. It can simultaneously handle several samples even of divergent nature and composition, supporting several analyses at a given time.

Steps involved in HPTLC

Step 1: Sample application.

The samples to be chromatographed are applied to the chromatogram layer. Volume precision and exact positioning are ensured by the use of a suitable instrument.

Step 2: Chromatogram development

The solvent (mobile phase) migrates the predetermined distance in the layer (stationary phase) by capillary action. In this process, the samples are separated into fractions. After evaporation of the mobile phase, the fractions remain stored on the layer.

Step 3: Chromatogram evaluation

The tracks (samples) are scanned in a densitometer with a light beam in the visible or ultraviolet range of the spectrum. Absorbance or fluorescence is measured by diffuse reflectance. Alternatively to classical densitometry, the chromatogram can be evaluated by video technology. Additional operations such as per or post-chromatographic derivatisation can be performed as required.

HPTLC has become the accepted term for layer which

- Area slightly thinner than conventional layers (0.20mm instead of 0.25 mm) and thus need less sample to show the same measuring result.
- Have a smaller mean grain size – 7 instead of 12-20 µm and in particular a closer grain size distribution than conventional layers.
- Hence give better resolution with a migration distance about 50% shorter-50mm as against -120mm.
- Have improved optical properties over conventional layers which give better accuracy during densitometric evaluation.

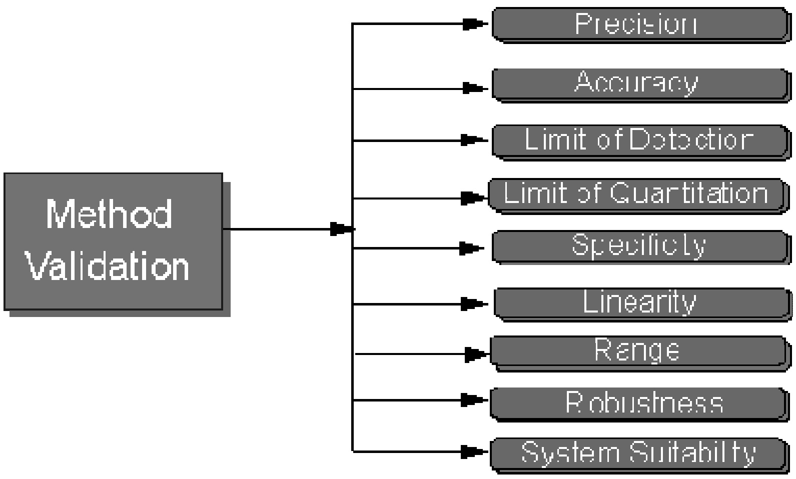
Comparative evaluation of HPTLC and HPLC techniques

1. Simultaneous analysis of standard and sample on the same chromatoplate is possible in HPTLC but it is not possible in HPLC
2. Low cost pre-coated HPTLC plates are available, whereas HPLC columns are very expensive.
3. Solvents need degassing in HPLC method but HPTLC doesn't need this.
4. Mobile phase consumption is extremely low in HPTLC and high in HPLC

5. No contamination can occur in HPTLC as fresh plates and mobile phase are used for each analysis but contamination occurs in HPLC.
6. Corrosive and UV absorbing mobile phases can be used in HPTLC but not in HPLC.
7. Substance sensitive to light and oxygen can create problem in HPTLC but it does not affect HPLC as it is closed system.

ANALYTICAL METHOD VALIDATION

Method validation can be defined as (ICH*)* "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics". Typical validation characteristics which should be considered are:



Accuracy

The accuracy of a method is the closeness of the measured value to the true value for the sample.

Accuracy criteria for an assay method is that the mean recovery will be 100±2% over the range of 80%-120% at the target concentration. For impurity method, the mean recovery will be within 0.1% absolute of the theoretical concentration or 10% relative, whichever is greater for impurities in the range of 0.1-2.5%v/w.

Precision

The precision of an analytical method is the amount of scatter in the results obtained from multiple analysis of a homogeneous sample. To be meaningful, the precision study must be performed using the exact sample and standard preparation procedures that will be used in the final method.

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as %RSD for the statistically significant number of samples. According to ICH, precision should be performed at three different levels:

a) Repeatability
b) Intermediate precision
c) Reproducibility

Repeatability is the result of method operating over a short interval under same conditions (Intra-assay precision) and it should be determined from a minimum of nine determinations covering the specified range of procedures (three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration.

Intermediate precision is the precision obtained when the assay is performed by multiple analysts, using multiple instruments, on multiple days, in one laboratory. Different sources of reagents and multiple lots of columns should also be included in this study. Intermediate precision results are used to identify which of the above factors contribute significant variability to the final result.

The last type of precision study is reproducibility, which is determined by testing homogeneous samples in multiple laboratories, often as part of inter laboratory crossover studies. The evaluation of reproducibility results often focuses more on measuring bias in results than on determining differences in precision alone. Statistical equivalence is often used as measures of acceptable inter laboratory results.

For an assay method, the instrument precision (intermediate precision) will be 1% and the intra-assay precision (Repeatability) will be 2% and for an impurity method, instrument precision will be 5% and intra-assay precision will be 10%.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weightings of synthetic mixtures of the drug product components, using the proposed procedure.

Linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration.

Five concentration levels from 50-150% of the standard solutions of the analyte are required to allow detection of curvature in the plotted data. Generally five concentrations ranging from 80-120% of standard concentration should be used in three replicates of each concentration.

Acceptability of linearity is often judged by correlation co-efficient and y-intercept of linear regression line for the response versus concentration plot. A correlation co-efficient of >0.999 is generally considered as evidence of acceptable fit of the data to the regression line. The Y-intercept should be less than a few percentage of the response obtained for the analyte at the target level. The recommended range for an assay method for content would be RSD ± 20% and the range for an assay/impurities combination method based on area % (for impurities) would be ± 20% of target concentration down to the limit of quantification of the drug substance or impurity.

Range

Range is the interval between upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy and linearity. Range of an analytical method is the concentration interval over which acceptable accuracy, linearity and precision are obtained.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Based on the standard deviation of the response and the slope, the detection limit (DL) may be expressed as

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = standard deviation of the response

S= slope of the calibration curve (of the analyte)

Quantification limit

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision, accuracy and reliability by the proposed method.

Based on the standard deviation of the response and the slope, quantitation limit may be expressed as,

$$QL = \frac{10\sigma}{S}$$

Where,

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

Ruggedness

Ruggedness is the degree of reproducibility of test results obtained by analysis of same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments, different lots of reagent, different elapse assay times, different assay temperatures, different days, etc. It is normally expressed as lack of influence on test results of operational and environmental variables of the analytical method.

Robustness

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

In the case of liquid chromatography, examples of typical variations are

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate.

System Suitability

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and the procedure are capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

System suitability parameters and recommendations:

S.No	Parameters	Recommendations
1	Theoretical plates (N)	>2000
2	Tailing factor (T)	≤ 2
3	Resolution (Rs)	> 2 between peak of interest and the closest eluting potential interference
4	Repeatability	RSD ≤ 1% for N ≥5 is desirable
5	Capacity factor (k^1)	> 2.0
6	Relative retention	Not essential as long as the resolution is stated

SYSTEM SUITABILITY PARAMETERS

[Lloyd, 1997; A.H. Beckett, and J.B. Stenlake, 2007]

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Column capacity factor (K_A)
- Resolution (R_s)
- Selectivity (α)
- Column efficiency (N) and
- Peak asymmetry factor (A_s)
- Tailing factor (T)
- Height Equivalent to a Theoretical Plate (HETP)

i) Column capacity factor (K_A)

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

Where,

V_A = Elution volume of A

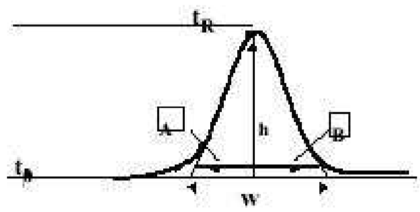
V_0 = Elution volume of a non retained compound (void volume).

At constant flow rate, retention times (t_A and t_o) can be used instead of retention volumes. Retention data is sometimes expressed, relative to a known internal standard (B). The ratio of retention times (t_A/t_B) can be used, but the ratio of adjusted retention times $\left(\frac{t_A - t_o}{t_B - t_o}\right)$ is better when data need to be transferred between different chromatographs.

The values of k' of individual bands increase or decrease with changes in solvent strength. In reversed phase HPLC, solvent strength increases with the increase in the volume of organic phase in the water / organic mobile phase. Typically an increase in percentage of the organic phase by 10 % by volume will decrease k' of the bands by a factor of 2-3.

RETENTION FACTOR or CAPACITY RATIO

$$k' = \frac{t_R - t_0}{t_0} \qquad k' = \phi \frac{C_s}{C_m}$$



ii) Resolution (R_s)

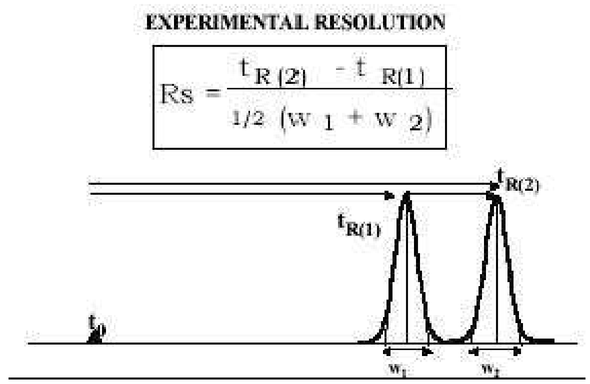
The resolution, R_s of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2.

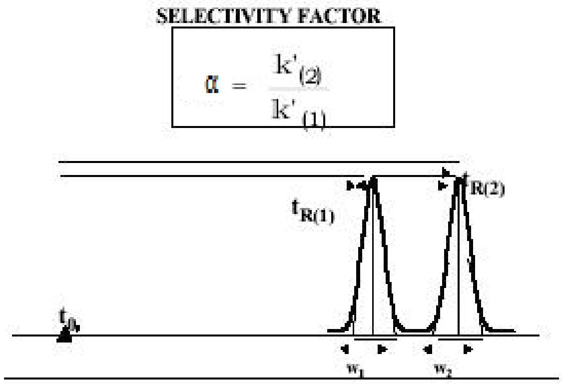


iii) Selectivity (α)

The selectivity (or separation factor), α is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak, respectively.



iv) Column efficiency

Efficiency (N) of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to100, 000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{W^2}$$

Where, Rt is the retention time and W is the peak width.

v) Peak asymmetry factor (A_s)

Peak asymmetry factor, A_s can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

Vi) Tailing factor (T)

A measure of the symmetry of a peak.

$$T = W_{0.05} / 2f$$

Where, $W_{0.05}$ -peak width at 5% height

f -distance from peak front to apex point at 5% height.

The accuracy of quantification decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak.

Limits-T </= 2

Vii) Height Equivalent to a Theoretical Plate (HETP)

A theoretical plate can be of any height, which decides the efficiency of separation. If HETP is less the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

$$\mathrm{HETP} = \frac{\text{Length of the column}}{\text{No. of the theoretical plates}}$$

STATISTICAL PARAMETERS

(Sundar Rao, 2006, Sanford Bolton, 1990; Kenneth, 2001; Mendham, 1994)

Linear regression

Linear regression a statistical technique that defines the functional relationship between two variable by best-fitting straight line. Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient ‘r’, then the best straight line through the data points has to be estimated. This can often be done be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares)

The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable.

To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N\,\Sigma xy - (\Sigma x)\,(\Sigma y)}{N\,\Sigma x^2 - (\Sigma x)^2}$$

And

$$c = \frac{(\Sigma y)\,(\Sigma x^2) - (\Sigma x)\,(\Sigma xy)}{N\,\Sigma x^{2} - (\Sigma x)^{2}}$$

Correlation coefficient

A measure of the strength of the relationship between two variables is provided by the coefficient of correlation, denoted by r, if the relationship between the two variables is of the linear form. It is also called the coefficient of linear correlation

Pearson’s correlation:

The correlation coefficient calculation for data values should be +1 or -1

Where the value of Correlation coefficient is +1 – positive

Correlation coefficient is -1 – negative

$$r = \frac{n\,\Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n\,\Sigma x_1^2 - (\Sigma x_1)^2]\,[n\,\Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Standard deviation

It is the square root of the average of the squared deviations of the observations. From the arithmetic mean, it is used for measures of dispersion.

It is denoted by

$$\text{Standard Deviation} = \sqrt{\frac{\Sigma(x-x)^2}{n-1}}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D}}{\overline{\text{x}}} \times 100$$

Where Σ = Sum of observations

$\bar{x}$ = Mean or arithmetic average (Σx / n)

x = Individual observed value

$x - \bar{x}$ = Deviation of a value from the mean

n = Number of observations

Standard error of mean (S.E)

The population of standard deviation is not given, but the size of s is large, so the sample standard deviation is representing the population of standard deviation.

$$S.E. = \frac{S.D}{\sqrt{n}}$$

Where,

S.D = Standard deviation

n = no. of observations

2. LITERATURE REVIEW

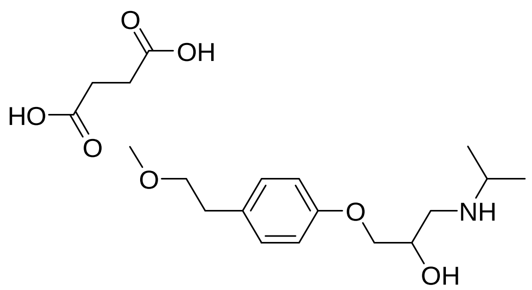
2. LITERATURE REVIEW

(The Merck Index, 2006, [www.en.wikipedia.org/wiki/Telmisartan, www.Free](www.en.wikipedia.org/wiki/Telmisartan) patents.com, *Martindale 2005, British Pharmacopoeia* 2009, *USP 2007)*

2.1. DRUG PROFILE

2.1.1. METOPROLOL SUCCINATE

Molecular Structure



Chemical Name

2-propanol, 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-, (±)-, butanedioate

Molecular Formula

$C_{19}H_{31}NO_7$

Molecular Weight

652.8 g/mol

Category

Cardio selective beta blocker

Description

White or almost white, crystalline powder.

Solubility

Free soluble in water, soluble in methanol.

Identification test

Melting point:

136-137ºC

IR spectrum:

Principle peak at wave numbers 1516.53, 1052.07, 1072.77, 1187.11 cm^{-1} show at fig-1

pH

7.0 - 7.6

pKa value

9.68

Storage

Stored in tightly closed containers in a cool and dry place. Protected from light.

Pharmacokinetics

Absorption:

Absorption of Metoprolol is rapid and complete.

Distribution:

Plasma levels following oral administration of conventional metoprolol tablet, however, approximate 50% of levels following intravenous administration indicating 50% first pass metabolism.

Excretion:

Elimination is mainly by biotransformation in the liver and plasms half life range from approximately 3 to 7 hours.

Pharmacological action

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Indications

Anti hypertension, anti angina, anti arrhythmic class (II)

Available Brand

Toprol-xl

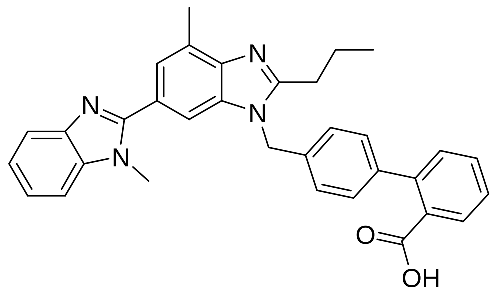
METPURE-XL

Adverse effects

Worsening of angina or myocardial infarction, worsening of heart failure, worsening of AV block

2.1.2. TELMISARTAN

Molecular Structure



Chemical Name

4′-[[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl] ethyl]

Biphenyl-2-carboxylic acid.

Molecular Formula

$C_{33}H_{30}N_4O_2$

Molecular Weight

514.6 g/mol

Category

Angiotensin II type 1 receptors

Description

White or slightly yellowish, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in methanol, sparingly soluble in methylene chloride. It dissolves in 1 M sodium hydroxide.

pH

3-9

pKa value

3.5, 4.1, 6.0

Identification test

Melting point:

261-263ºC

IR spectrum:

Principle peak at wave number 3056.79, 2928.11, 1696.15, 758.33 cm^{-1} show fig-2

Storage

Stored at 25°C (77°F) in tightly closed containers in a cool and dry place

Pharmacokinetics

Absorption:

Oral administration, peak concentrations (C_{max}) of telmisartan are reached in 0.5-1 hour after dosing.

Distribution:

Food slightly reduces the bioavailability of telmisartan. The absolute bioavailability of telmisartan is dose dependent. At 40 and 160 mg the bioavailability was 42% and 58%, respectively.

Excretion:

Eliminated unchanged in feces via biliary excretion; only minute amounts were found in the urine.

Pharmacological action

Angiotensin II is the principle pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Telmisartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland.

Blockade of the renin-angiotensin system with ACE inhibitors, which inhibit the biosynthesis of angiotensin II from angiotensin I, is widely used in the treatment of hypertension

Indications

Anti hypertension

Available Brand

Micardis

TELMA

TELSAR

TETAN

Adverse effects

Upper respiratory tract infection, Back pain, Sinusitis, Diarrhea, Pharyngitis

2.2. REPORTED METHODS

2.2.1. METOPROLOL SUCCINATE:

1. **Vachhani kevin H *et al.*, (2011)**

   "Development of validation of first order derivative spectrophotometric method for simultaneous estimation of Metoprolol succinate and olimesartan medoxomil in tablets".

   Method - First order derivative spectrophotometric method

   Solvent - Methanol

   λmax -204.6nm,275.6nm

2. **Chandra Bose R.J.*et al., (*2011)**

   "Validated Rp-Hplc meth0d for the simultaneous estimation of Ramipril and Metoprolol tartrate in Bulk and Tablet dosage form".

   Elution - Isocratic

   Stationary phase - Hypersil C_{18} column (150 mmx4.6 mm,5µm)

   Mobile phase -Acetonitrile: Methanol:10mM acetate buffer pH 5 (30:50:20 v/v)

   Detection - UV detector,

   Wavelength -210nm

3. **Sagar B. Wankhede *et al., (*2011)**

   "Stability indicating HPTLC method for quantitative determination of Atorvastatin calcium and Metoprolol succinate in capsules".

   Stationary phase - silica gel60 F_{254} (0.2 mm thickness)

Mobile phase - Toluene: Methanol: Ethyl acetate: Glacial acetic acid

7 : 1.5 : 1 : 0.5 (v/v)

Quantification - Densitometry at 276 nm.

Wavelength -254nm

4. **Mitesh D *et al., (*2010)**

"Optimization and Establishment of a validated stability-indicating HPLC method for study of the stress degradation behavior of Metoprolol succinate".

Elution - Isocratic

Stationary phase - C-18 column

Mobile phase -sodium dihydrogen phosphate buffer: Acetonitrile

(70:30 v/v)

Detection - UV detector,

Wavelength -274nm

5. **Raja Kumar Seshadri *et al., (*2010)**

"Simultaneous Quantitative determination of Metoprolol, Atorvastatin and Ramipril in capsules by a validated stability-indicating RP-UPLC method".

Elution - Isocratic

Stationary phase - Zorbax^{R} XDC-C_{18} (4.6mmX50mm, 1.8µm)

Mobile phase - Buffer:Acetonitrile (50:50 v/v)

Detection - UV detecter,

Wavelength -210nm

6. Rajendra Kakde *et al., (*2009)

"High performance thin layer chromatographic method for simultaneous estimation of Metoprolol succinate and amlodipine besylate in pharmaceutical preparations".

Stationary phase - silica gel60 F_{254} (0.2 mm thickness)

Mobile phase - Methanol: Ethyl acetate: Water: Toluene: 25% Ammonia

1.5 : 5.0 : 0.3 : 3.0 : 0.3 (v/v)

Quantification - Densitometry at 236 nm.

7. Moreshwar N. kulkarni *et al., (*2009)

"Development and validation of spectrophotometric method for determination of Metoprolol succinate".

Method - Maximum absorption

Solvent - Distilled water, phosphate buffer 6.8, 0.1M HCl

λmax -224nm

8. Mitesh D Phale *et al., (*2009)

"A validated and simplified RP-HPLC of Metoprolol succinate from bulk drugs".

Elution - Isocratic

Stationary phase - RP spherisorb C-18 (250 x4.6 mm. 10µm)

Mobile phase - Acetonitrile: methanol: 10mM aqueous phosphate buffer

(20:20:60 v/v)

Detection - UV detecter.

9. Singh Brijesh *et al., (*2009)

"Development of Reverse-phase HPLC method for simultaneous analysis of Metoprolol succinate and Hydrochlorothiazide in a tablet formulation".

Elution - Isocratic

Stationary phase - C-18 column

Mobile phase - 50mM di-sodium hydrogen phosphate: methanol: Acetonitrile (525:225:250 v/v)

Detection - UV detector,

Wavelength -222nm

10. Senthamil selvan P. *et al., (*2008)

"Chromatography-tandem mass spectrometry method for the simultaneous quantification of Metoprolol succinate and simuvastatin in human plasma".

Elution - Isocratic

Stationary phase - C-18 column

Mobile phase - Acetonitrile: 0.5% formic acid P^H 3.5 (90:10 v/v)

Detection - Mass detector,

Mode -positive

11. Sathe S.R *et al., (*2008)

"Development of HPTLC method for the estimation of Metoprolol succinate in bulk and in tablet dosage form".

Stationary phase - silica gel60 F_{254} (0.2 mm thickness)

Mobile phase - Toluene: Methanol: Triethylamine

3 : 0.5 : 0.3

Quantification - Densitometry at 274 nm.

2.2.2. TELMISARTAN

1. **Rekha Gangola** ***et al., (*****2011)**

   "Spectrophotometric simultaneous determination of Hydrochlorothiazide and Telmisartan in combined dosage form".

   Method - simultaneous equation

   Solvent - 0.1M NaoH

   λmax -273nm,295nm

2. **Sagar Tatane** ***et al., (*****2011)**

   "Development of UV spectrophotometric method of Telmisartan in Tablet formulation".

   Solvent - Methanol: Water (70:30)

   λmax -230nm

3. **Rekha Gangola** ***et al., (*****2011)**

   "Spectrophotometric simultaneous determination of Hydrochlorothiazide and Telmisartan in combined dosage form by dual wavelength method".

   Method - Dual wavelength method.

   Solvent - 0.1M NaoH

   λmax -258nm,299nm,316nm,326nm

4. **Ilango K** ***et al., (*****2011)**

   "Simultaneous estimation of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage form".

   Method - simultaneous equation

   Solvent - Methanol

   λmax - 296nm,270nm

5. Kumbhar S.T. *et al., (*2011)

"Visible spectrophotometric determination of Telmisartan from urine".

Method - colorimetric method

Solvent - Methanol

Reagent - Thionyl chloride

λmax -427nm

6. Vijay kumar G *et al., (*2011)

"Validated RP-HPLC method for the esatimation of Telmisatan in serum samples".

Elution - Isocratic

Stationary phase - Equsil (250X 4.6 mm, 5µ)

Mobile phase - Buffer: Acetonitrile

(35:65 v/v)

Detection - UV detector,

Wavelength - 282nm

7. Patel Amit R *et al., (*2011)

"Method development, validation and sability study for simultaneous estimation of Telmisartan and indapamide by Reverse phase-High performance liquid chromatography in pure and marked formulation".

Elution - Isocratic

Stationary phase - amazone C-18 (150X 4.6 mm, 5µ)

Mobile phase - Buffer: Acetonitrile: Methanol

(45:25:30v/v)

Detection - UV detector,

Wavelength - 285nm

8. Santaji Nalwade *et al., (*2011)

"Rapid simultaneous determination of Telmisartan, Amlodipine besylate and Hydrochlorothiazide in combined poly pill dosage form by stability – indicating ultra performance liquid chromatography".

Elution - Isocratic

Stationary phase - C-18 (100X 2.1 mm, 1.7µ)

Mobile phase - Sodium per chlorate buffer: Acetonitrile: (90:10v/v)

Detection - UV detector,

Wavelength - 271nm, 237nm

9. Ramesh J *et al., (*2011)

"Development and validation of HPTLC method for the simultaneous estimation of Atorvastatin and Telmisartan in combined dosage form".

Stationary phase - silica gel60 F_{254} (10cm x 10 cm)

Mobile phase - Chloroform: Benzene: Methanol: Glacial acetic acid

6 : 3 : 1.0 : 0.1 (v/v/v/v)

Quantification - Densitometer at 265 nm.

10. Patel V.A *et al., (*2011)

"Development and validation of HPTLC method for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form".

Stationary phase - silica gel60 F_{254} (10 cm x 10 cm)

Mobile phase - Acetone: Benzene: Ethyl acetate: Glacial acetic acid

5.0 : 3.0 : 2 : 0.03 (v/v)

Quantification - Densitometry at 210 nm, 296 nm.

11. Lakman V. Portale *et al., (*2010)

"A validated stability indicting HPTLC method for simultaneous estimation of Ramipril and Telmisartan".

Stationary phase - silica gel60 F_{254} (10 cm x 10 cm)

Mobile phase - Methanol: Chlorform

1 : 6 (v/v)

Quantification - Densitometry at 210 nm.

12. patil U.P *et al., (*2010)

"A validated densitometric method for analysis of Telmisartan and Atorvastatin calcium in fixed dose combination".

Stationary phase - silica gel60 F_{254} (10 cm x 10 cm)

Mobile phase - Toluene: Methanol

7 : 3 (v/v)

Quantification - Densitometry at 280 nm.

13. Lakshmi K.S. *et al., (*2010)

"Stability indicating HPTLC method for simultaneous estimation of Telmisartan and Ramipril in tablets".

Stationary phase - silica gel60 F_{254} (10 cm x 10 cm)

Mobile phase - Toluene: Acetonitrile: Formic Acid: Water:

5 : 5 : 0.3 : 1 (v/v)

Quantification - Densitometry at 212 nm.

14. Popat B. mohite *et al., (*2010)

"Simultaneous estimation of Ramipril and Telmisartan in tablet dosage form by spectrophotometry".

Method - multicomponant method

Solvent - 0.2M H_2SO_4

λmax -205nm,291nm.

15. Kalyankar T.M. *et al., (*2010)

"A rapid colorimetric method for the estimation of ammonia in Telmisartan in bulk and solid dosage form".

Method - colorimetric method

Solvent - Water

λmax - 425nm.

16. Vijayamirtharaj R. *et al., (*2010)

"Development and validation of RP-HPLC method for the simultaneous estimation of Telmisartan of Atorvastatin calcium in tablet dosage forms".

Elution - Isocratic

Stationary phase - Phenomenex C-18 (250 X 4.6 mm, 5µ)

Mobile phase - Acetonitrile: Buffer (0.01M potassium dihydrogen phosphate)

(65:35v/v)

Detection - UV detector,

Wavelength - 250nm

17. Yogesh Gupta *et al., (*2009)

"Isocratic RP-HPLC method development and validation for the simultaneous estimation of Ramipril and Telmisartan in tablet dosage form".

Elution - Isocratic

Stationary phase - ODS column hypersil (250 X 4.6 mm, 5µ)

Mobile phase - Acetonitrile: Buffer (3.9 gm sodium dihydrogen phosphate)

(65:35v/v)

Detection - UV detector,

Wavelength - 210nm

18. Wankhede SB *et al., (*2009)

"RP-HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form".

Elution - Isocratic

Stationary phase - ODS column hypersil C-18(25cm X 4.6 mm, 5µ)

Mobile phase - Acetonitrile: Buffer (0.05M Potassium dihydrogen phosphate

(60:40v/v)

Detection - UV detector,

Wavelength - 271nm

3. AIM AND PLAN OF WORK

3.1 AIMOF WORK

The prime importance of drug analysis is to gain information about the qualitative and quantitative composition of substance and chemical species, that is to find out what a substance is composed of and exactly how much. This information guides development of the manufacturing operations and therapeutic action of drugs.

Standard analytical procedure for newer drugs or its formulation may not be available in Pharmacopoeias. Hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

Metoprolol succinate and Telmisartan is a newer combination launched in Indian market. Metoprolol succinate and telmisartan drugs are used for treatment of hypertension. But there are several methods were reported for the estimation of metoprolol succinate and telmisartan individually as well as in combination with some other drugs. But there are no methods were reported for the estimation of these drugs in combined dosage forms without prior separation.

Hence the present study aims to develop simple, precise and accurate methods for the determination of metoprolol succinate and telmisartan by simple UV methods and RP-HPLC, HPTLC method in pure and tablet dosage form.

3.2 PLAN OF WORK

- The extensive survey of literature for metoprolol succinate and telmisartan regarding their physiochemical properties, pharmacological properties and analytical methods. This formed the basis for the development of methods.

- Selection and collection of metoprolol succinate and telmisartan working standard for analysis.
- Identification of working standard by IR spectroscopy, Melting point tests.
- Selection of suitable solvent for quantitative extraction of drug present in the tablet dosage form. The solvent should be readily available, economical and of analytical grade for UV-spectroscopy and HPLC grade for RP-HPLC and HPTLC should not chemically interact with the compound of interest and its structural characteristics.
- Selection of suitable method for analysis depending upon the spectral characteristics of the drug.
- Selection of suitable wavelength for rapid, accurate, precise and simple UV spectroscopic methods development.
- Development of rapid and accurate RP-HPLC method by using UV detector.
- Development of rapid and accurate HPTLC method by using UV detector.
- Analysis of Tablet by the UV Spectroscopy and RP-HPLC, HPTLC Methods.
- Statistical analysis of developed analytical methods.
- Validation of analytical methods as per the ICH guidelines

4. MATERIALS AND METHODS

4.1. MATERIALS USED

4.1.1. DRUGS

Metoprolol succinate and Telmisartan were generously gifted by SKN Pharmaceuticals, Pondicherry and APEX Pharmaceuticals, Chennai.

4.1.2. FORMULATION:

METOSARTAN-25 formulation, Sun-Pharma industries, containing 25 mg of Metoprolol succinate and 40 mg of Telmisartan was purchased from a local pharmacy.

4.1.3. REAGENTS & CHEMICALS

All the chemicals used were of analytical reagent grade and HPLC grade procured from Qualigens, India Ltd. The chemicals used for the study were

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Water (HPLC grade)

Chloroform (Analytical grade)

Methanol (Analytical grade) and

Formic acid (Analytical grade)

Hydrochloric acid (Analytical grade)

4.1.4. INSTRUMENTS SPECIFICATIONS

1) Shimadzu AX – 200 digital balances: (Shimadzu instruction manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

2) Shimadzu UV – Visible spectrophotometer: (Shimadzu instruction manual)

Model : Shimadzu, UV-1700, Pharmaspec. Cuvetts: 1 cm matched quartz cells

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment. Mechanism
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10 g L^{-1}) 0.04% or less (340 nm: $NaNo_2$ 50 g L^{-1})
Measurement	190~1100 nm

wavelength range	
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)
Temperature/Humidity	Temperature range : 15 to 35°C Humidity range : 35 to 80% (15 to below 30° C) 35 to 70% (30 to 35° C)

3) High Performance Liquid Chromatography: (Water TM 486- Tunable absorbance detector)

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength	190 to 500 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µ L^{-1}
Operating temperature range	4 to 35° C (39 to 104° F)

Recording range	0.0001 to 4.000 AUFS
Operating	4 to 35° C / 75 %

Pump Specifications-Water 510 HPLC pumps	
Pump type	Double reciprocating plunger pump
Pumping method	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating	4 to 35° C (39 to 104° F)

4) High performance thin layer liquid chromatography:

Pre Coated Silica Gel Plates:

Silica gel 60-254 aluminum sheets were procured from, Merck, Drarm stadt,Germany.

CAMAG HPTLC System with Linomat 5 Applicator,

Camag TLC Scanner 3 and Wincats software

5) Sonica ultra sonic cleaner- model 2200 MH

6) ELICO – pH meter model L1610

7) Micropipette

8) Melting point apparatus - Guna enterprises Chennai.

4.2 METHODS EMPLOYED

The methods employed for the simultaneous estimation of Metoprolol succinate and Telmisartan are

4.2.1. UV Spectrophotometric method

a) Simultaneous Equation Method

b) First order derivative spectroscopic method

c) Area under the curve method and

d) Geometric correction method.

Identification of Drugs

Metoprolol succinate and Telmisartan working standards were identified from their Infra- Red spectrum shown in fig-1 & 2, melting point test shown in Table 3.

Selection of solvent

The solubility of drugs were carried out using various polar to non polar solvents. The Solubility profiles of the drugs were determined as per the standards mentioned in the Indian Pharmacopoeia show in Table-1&2. The common solvent was found to be methanol and 0.1 M HCl. For the analysis of metoprolol succinate and telmisartan by the proposed method, 0.1 M HCl were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, solubility, and stability factor and cutoff wavelength.

Preparation of standard stock solution of Metoprolol succinate

Pure raw material of metoprolol succinate 10 mg was accurately weighed and dissolved in 0.1 M HCl to produce 1000 μg mL^{-1}. From this 2 mL of the solution was transferred in to the 100 mL volumetric flask and made up to required volume with 0.1 M HCl to get the concentration 20 μg $mL^{-1.}$ It is used as a working standard.

Preparation of standard stock solution of Telmisartan

Pure raw material of telmisartan 15 mg was accurately weighed and dissolved in 0.1 M HCl to produce 1000 μg mL^{-1}. From this 2 mL of the solution was transferred in to the 50 mL volumetric flask and made up to required volume with 0.1 M HCl to get the concentration 60 μg $mL^{-1.}$ It is used as a working standard.

Preparation of biological sample solution

Pure raw material of metoprolol succinate 25 mg and telmisartan 40 mg was accurately weighed and dissolved in artificial urine to produce 1000 μg mL^{-1}. From this 2 mL of the solution was transferred in to the 50 mL volumetric flask and made up to required volume with 0.1 M HCl to get the concentration 40μg mL^{-1}, 60 μg $mL^{-1.}$ It is used as a working sample solution.

Artificial urine preparation:

To 1.5 liters of distilled water added 36.4 g of urea and mix until all the crystals were dissolved. Then added 15.0 g of sodium chloride, 9.0 g of potassium chloride and 9.6 g of sodium phosphate; mix until the solution was clear. Check the pH with indicator paper or a pH meter to ensure the pH is within the 5 to 7 pH range for normal urine; if the solution is out of this pH range the pH may be lowered with 1N hydrochloric acid or raised with 1N sodium hydroxide.

Selection of wavelengths

The selection of wavelength for the estimation of metoprolol succinate and telmisartan, a suitable standard solution to contain 10 $\mu g\ mL^{-1}$ of metoprolol succinate and telmisartan were prepared individually and scanned in the entire range from 200-400 nm. From the overlaid spectra, the two wavelengths 275nm for metoprolol succinate and 228.5nm telmisartan were selected for the construction simultaneous equation method.

For Derivative Spectroscopic method, the zero order spectrums were derivatised to their first order. In that 269 nm was selected for the estimation of metoprolol succinate, which is zero crossing for telmisartan and 243 nm, was selected for the estimation of telmisartan which is zero crossing for metoprolol succinate.

For Area under the curve method, the range of the areas were selected for both the drugs from their zero order spectra's, 227.5-214 nm were chosen for the estimation of metoprolol succinate and 303-278.5 nm were chosen for the estimation of telmisartan.

For Geometric correction method, the three wavelengths were selected for both the drugs from their zero order spectra's, 217,225,232 nm for the estimation of metoprolol succinate and 267,275,283 nm for the estimation of telmisartan.

Stability studies

The stability studies were performed by measuring the absorbance of same solution at different time intervals. It was observed that metoprolol succinate and telmisartan in the specified solvent were stable for more than 4 hours at their wavelength maxima.

Linearity

The linearity studies for the simultaneous equation method, Area under the curve method Geometric correction method, were performed at 275 nm, 227.5-214nm, (217,225,232nm) and 228.5 nm, 303-278.5nm, (267,275,283nm) in the concentration range of 2-10 $\mu g\ mL^{-1}$ for metoprolol succinate and 3.2-16 $\mu g\ mL^{-1}$ for telmisartan.

For Derivative Spectroscopic method, the $\Delta A/\Delta\lambda$ values were measured in the first order derivative mode. Linearity was performed at 269 nm and 243 nm in the concentration range of 10-50 $\mu g\ mL^{-1}$ for metoprolol succinate and 16-80 $\mu g\ mL^{-1}$ telmisartan.

Preparation of calibration curve

The calibration curves were constructed by plotting absorbance Vs concentration for simultaneous equation method, $\Delta A/\Delta\lambda$ Vs concentration for first order derivative method, Area Vs concentration for area under the curve method, Corrected absorbance Vs concentration for Geometric correction method.

Quantification of formulation

Simultaneous equation method, Area under the curve method.

Twenty tablets of (METOSARTAN-25) containing 25 mg Metoprolol succinate and 40 mg of Telmisartan) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of metoprolol succinate weighed and transferred into 25 ml volumetric flask and added a minimum quantity of 0.1M HCl to dissolve the substance and made up to the volume with the same (1000 μg mL^{-1}). The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 4 mL of the solution was transferred into a 50 mL volumetric flask and made up to the required volume with 0.1M HCl to get the concentration 80 μg/ml. 0.5 mL were transferred into a series of 10 mL volumetric flasks and made up to the mark with 0.1M HCl to get the concentration of 4 μg mL^{-1} solution of metoprolol succinate which is also contains 6.4 μg/ml of telmisartan theoretically. The absorbance measurements were made six times for the formulations at 275 nm, 227.5-214 nm and 228.5 nm, and 303-278.5 nm. The amount of metoprolol succinate and telmisartan were found by constructing Simultaneous equation method, for area under the curve method the area were substituted in the simultaneous equation to found the amount.

For Derivative Spectroscopic Method:

Twenty tablets of (METOSARTAN-25 containing 25 mg Metoprolol succinate and 40 mg of Telmisartan) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of metoprolol succinate weighed and transferred into 25 mL volumetric flask and added a minimum quantity of 0.1M HCl to dissolve the substance and made up to the volume with the same (1000 $\mu g\ mL^{-1}$). The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 20 mL of the solution was transferred into a 50 mL volumetric flask and made up to the required volume with 0.1M HCl to get the concentration 80 $\mu g\ mL^{-1}$. 0.5 mL were transferred into a series of 10 mL volumetric flasks and made up to the mark with 0.1M HCl to get the concentration of 20 $\mu g\ mL^{-1}$ solution of metoprolol succinate which is also contains 32 $\mu g\ mL^{-1}$ of telmisartan theoretically. The $\Delta A/\Delta\lambda$ measurements were made six times for the formulations at 269 nm and 243 nm. The amount of metoprolol succinate and telmisartan were found by constructing Area under curve method.

For Geometric correction method:

Twenty tablets of (METOSARTAN-25 containing 25 mg Metoprolol succinate and 40 mg of Telmisartan) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of metoprolol succinate weighed and transferred into 25 mL volumetric flask and added a minimum quantity of artificial urine to dissolve the substance and made up to the volume with the same (1000 $\mu g\ mL^{-1}$). The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the extracted solution, further dilutions were made by diluting 4 mL of the solution was transferred into a 50 mL volumetric flask and made up to the required volume with 0.1M HCl to get the concentration 80 μg mL^{-1}. 0.5 mL were transferred into a series of 10 mL volumetric flasks and made up to the mark with 0.1M HCl to get the concentration of 4 μg mL^{-1} solution of metoprolol succinate which is also contains 6.4 μg mL^{-1} of telmisartan theoretically. The absorbance measurements were made six times for the formulations at (217nm, 225nm, 232nm) and (267nm, 275nm, 283nm). The same procedures were repeated without urine sample. Metoprolol succinate and telmisartan were found by constructing Geometric correction method and the amounts were found by substituted the corrected absorbance in the simultaneous equation method.

Recovery studies

The recovery experiment was done by adding known concentrations of metoprolol succinate and telmisartan working standard to the 50% pre analyzed formulations. Standard metoprolol succinate and telmisartan raw material solutions were prepared in 0.1 M HCl. Suitable amount of standard solutions containing concentrations of metoprolol succinate and telmisartan equivalent to 80 %, 100 % and 120% of the test concentration were added to the 50% pre analyzed formulation. The absorbances for simultaneous equation method, aera for area under curve method, $\Delta A/\Delta \lambda$ for derivative method, corrected absorbances for geometric correction method of the resulting solutions were measured at the selected wavelengths for the determination of metoprolol succinate and telmisartan for the four methods. The amount of each drug recovered was calculated. The procedure was repeated for three times.

Validation of developed method

Linearity and Range

From the calibration graphs plotted, metoprolol succinate and telmisartan shows the linearity between 2-10 μg mL^{-1}, 3.2-16μg mL^{-1} for the Simultaneous equation, Geometric correction method and Area under Curve method, 10-50 μg mL^{-1}, 16-80μg mL^{-1} for Derivative spectroscopic method respectively.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. The % RSD was calculated and tabulated.

Precision

The repeatability of the method was confirmed by the analysis of tablets repeated for 6 times with the same concentration. The amount of each drug present in the tablets was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of tablets was repeated three times in the same day and on three successive days. The amount of drugs was determined and the percentage RSD was calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of the tablets done by the different analysts. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of response.

4.2.2 RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following

S.No	Mobile phase	Observation
1	Acetonitrile: water (50: 50% v/v)	Telmisartan peak splited
2	Acetonitrile: Methanol: Water(60:30:10 v/v)	No markable resolution between peaks
3	Acetonitrile : Methanol: Water(40:40:10 v/v)	No markable resolution between peaks
4	Acetonitrile : Buffer pH 5	Both peaks were not sharp.

From the above information, in the mobile phase of Acetonitrile: Phosphate buffer pH 3 (25: 75 v/v), these two drugs were eluted with sharp peak and better resolution. Hence this mobile phase was used.

Selection of Detection wavelength

Solutions of Metoprolol succinate and Telmisartan (10 μg mL^{-1}) were prepared in the mobile phase and scanned in the UV region of 200 – 400 nm and recorded the spectrums. From the overlain spectra it was found that the two drugs have marked absorbance at 230 nm and can be effectively used for estimation of two drugs without interference. Therefore 230 nm was selected as detection wavelength for estimation of both the drugs by RP – HPLC method with an Isocratic elution technique.

Stability of sample solutions

Solutions of Metoprolol succinate and Telmisartan (10 μg mL^{-1}) were checked for their stability of absorbance at 230 nm and it was found that two drugs are stable for approximately 3 hour.

Optimized chromatographic conditions

The following optimized conditions were employed for analysis of Metoprolol succinate and Telmisartan by Isocratic RP – HPLC method.

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (250 mm × 4.6 mm i.d. 5μ)
Mobile phase	-	Acetonitrile: Phosphate buffer pH 3
Proportion of mobile phase	-	(25: 75 v/v)
Detection wavelength	-	230 nm

Flow rate - 1.0 mL min^{-1}

Temperature - Ambient

Sample load - 20 μL

Operating pressure - 1000 psi

Preparation of stock solution

Weighed accurately the tablet equivalent to 25 mg of metoprolol succinate in a 25 mL volumetric flask and dissolved in methanol, after dissolution, the volume was made up to the mark with methanol. It contains 1000 μg mL^{-1} Metoprolol succinate and 1600 μg mL^{-1} of Telmisartan.

Preparation of Calibration curve

From the stock solution, pipetted out 0.3mL, 0.4mL, 0.5mL, 0.6mL, and 0.7mL of solutions to 5 separate 10 mL flasks and made up to the volume with mobile phase, solutions containing the concentrations of 30, 40, 50, 60, and 70 μg mL^{-1} of metoprolol succinate and 48, 64, 80, 96 and 112 μg mL^{-1} telmisartan. After acquiring study baseline, the solutions were injected and the chromatograms were recorded at 230 nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Metoprolol succinate and Telmisartan in tablets

Estimation of metoprolol succinate and telmisartan in tablets by RP-HPLC was carried out using optimized chromatographic conditions. Weighed accurately the tablets equivalent to 25 mg of metoprolol succinate in a 10 mL volumetric flask and dissolved in methanol, after dissolve, the volume was made up to the mark with methanol. The content was sonicated for 15 minutes, and filtered through Whatmann filter paper No. 41. From the clear solution, 0.5 mL was transferred into six 10 ml (50 $\mu g\ mL^{-1}$ of metoprolol succinate and 80 $\mu g\ mL^{-1}$ of telmisartan) volumetric flasks individually and made up to the mark with mobile phase. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for one hour, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Experiments

a) Preparation of Metoprolol succinate and Telmisartan raw material stock solution

An accurately weighed quantity of 25 mg of metoprolol succinate and 40 mg of telmisartan were transferred into 25 mL volumetric flasks separately and added sufficient methanol to dissolve the substance and made up to the mark with the same solvent, it contains metoprolol succinate solution (50 $\mu g\ mL^{-1}$) and telmisartan solution (80 $\mu g\ mL^{-1}$).

Procedure

To each 0.5 mL of preanalysed sample solution (50 μg mL^{-1} of Metoprolol succinate and 80 μg mL^{-1} of Telmisartan), added 0.4 mL, 0.5 mL and 0.6 mL of metoprolol succinate and telmisartan working standard stock solutions into 10 mL volumetric flasks and made up to the mark with mobile phase and performed the recovery as described under assay shown in fig -40 to 42. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

System suitability studies

The system suitability parameters comply as per ICH guidelines and USP. The parameters like capacity factor, tailing factor, asymmetry factor, number of theoretical plate and resolution were calculated.

4.2.3. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

The various steps involved in HPTLC are

- Selection of chromatographic layer
- Sample and standard preparation
- Layer pre-conditioning
- Application of sample and standard
- Chromatogram development
- Detection of spots
- Scanning

Instrument Specification Pre coated Silica Gel Plates

Silica gel 60-254 aluminum sheets were procured from, Merck, Drarm stadt, Germany.

Stationary Phase

Plate size(X x Y)	- 20 x10 cm
Material	- Silica gel 60-254 aluminum sheets
Pre washing	- No
Modification	- No

Instrument

CAMAG Automatic TLC sampler 4 (ATS4) with Linomat 5 Applicator, CAMAG TLC Scanner 3 and Wincats software.

CAMAG HPTLC System Chromatography Parameters

Spray gas	-	Nitrogen
Sample solvent type	-	Methanol
Dosage speed	-	50 nL/s
Pre dosage volume	-	0.2 µL

Sequence

Syringe size	-	25 µL
Application type	-	Band
Band length	-	8 mm

Development – Glass Tank

Chamber type	-	Twin trough chamber 20 x10 cm
		Pre-conditioning
Mobile phase	-	Chloroform: Methanol: Formic acid
		(85:15:2.5% v/v)
Solvent front position	-	50.0 mm
Volume	-	10.0 mL
Drying device	-	Oven
Temperature	-	60°C
Time	-	5 minutes

Detection

CAMAG TLC Scanner 3 TLC scanner 3

Display scaling - Automatic

Reagents and Chemicals

- Chloroform HPLC grade
- Methanol gradient HPLC grade
- Formic acid HPLC grade
- Silica gel 60 F_{254} aluminum sheets
- Active pharmaceutical ingredients and formulation

Optimized Chromatographic Conditions

Sample prepared in	:	Methanol
Stationary phase	:	Silica gel 60 F_{254} aluminum sheets
Mobile phase	:	Chloroform: Methanol: Formic acid

(85:15:2.5% v/v)

Scanning wavelength : UV 233 nm

Development mode : Ascending mode

Choice of Mobile Phase

Initial trials were made with prepared TLC plates and pre- coated sheets, the spots were identified using shorter wavelength in UV chamber and they are confirmed by scanning in HPTLC scanner and the individual standards are also spotted to determine the R_f of each drugs.

TRIAL NO	MOBILE PHASE	RATIO
1.	Ethyl acetate: Methanol	80:20
2.	Ethyl acetate: Methanol : 10% Glacial acetic acid	80:20:1
3.	Ethyl acetate : n-Butanol : 10% Glacial acetic acid	60:20:20
4.	Toluene : Isopropyl alcohol :Water	50: 40:10
5.	Methanol : Chloroform	90:10

The mobile phase chosen after trial was Chloroform: Methanol: Formic acid (85:15:2.5% v/v) due to its better resolution.

Preparation of Standard Stock Solution

A standard stock solution of metoprolol succinate and telmisartan was prepared by dissolving in methanol and produced 1000 μgmL^{-1} and the solution was used to establish linearity

Sample Application

The sample was applied to the chromatographic plate and developed above said mobile phase, the volume, precision and exact positioning was ensured by the use of a suitable instrument and the plates were then air dried and placed into twin trough chamber for development of chromatogram.

Chromatogram Evaluation

After development, the plates were dried using nitrogen gas and the different tracks in the chromatographic plate were scanned in a densitometer with a light beam of UV range. The absorbance was measured by reflectance. The peak area obtained and the R_f value was noted.

Evaluation of Linearity

From stock solution of 1000 μgmL^{-1}, 1mL for metoprolol succinate, 1mL for telmisartan was pipette out in to a 50 mL volumetric flask and dissolved in methanol to get a concentration of 20 $\mu gmL^{-1,}$ 32 μgmL^{-1}. From this, a final concentration of metoprolol succinate and telmisartan ranging from 1 μgmL^{-1} - 5 μgmL^{-1} and 1.6 μgmL^{-1} – 8 μgmL^{-1} was obtained and this solution was spotted on a pre-coated TLC plates and developed as per the procedure discussed the peak area obtained for the different concentration.

Calibration Graph

A graph of peak area against concentration was constructed for metoprolol succinate and telmisartan in the concentration range of 1 μgmL^{-1} - 5 μgmL^{-1} and1.6 μgmL^{-1} – 8 μgmL^{-1}. It was found to be linear.

Quantification of formulation

Twenty tablets of each formulation (METOSARTAN-25) containing 25 mg of metoprolol succinate and 40 mg of telmisartan were accurately weighed and powdered. The powdered tablet equivalent to 25 mg metoprolol succinate and 40 mg of telmisartan was transferred in to a 25 mL volumetric flask, added 20 mL of HPLC grade methanol and sonicated for 20 min, then shaken vigorously for few min and finally made up to the mark with HPLC methanol. The above solution was collected by filtering it through Whatmann filter paper No.41. From the filtered solution, 1mL to 50 mL volumetric flask and further diluted 1mL to 10 mL volumetric flask. From the solution 2 μgmL^{-1} spot was spotted on a Silica gel 60-F254 aluminum sheets are allowed to develop in Twin Trough Chamber 20 × 10 cm using Chloroform: Methanol: Formic acid (85:15:2.5% v/v) as a mobile phase, the solvent front position is noted, the plates are then removed and allowed it to dry in oven at 60˚C for 5 min. The spots are then detected using Camag TLC Scanner 3 and the peak area obtained at the detecting wave length 233 nm, the amount of metoprolol succinate and telmisartan was calculated using the linear regression equation.

Recovery studies

The recovery experiment was done by adding known concentrations of metoprolol succinate and telmisartan working standard to the pre analyzed formulations. The results were determined.

5. RESULTS AND DISCUSSION

5.1. RESULTS AND DICUSSION

Simultaneous estimation of multiple drug formulations have advantage over the other methods, since it is less time consuming and less usage of solvent. Four simple, rapid, precise and accurate UV Spectroscopic methods, isocratic RP – HPLC method and HTPLC method were developed and validated for the estimation of Metoprolol succinate and Telmisartan in pure form, biological sample and in combined tablet dosage form. The methods employed are.

1. UV Spectrophotoscopic methods
    i. Simultaneous Equation method
    ii. Derivative Spectrophotometric method
    iii. Area under the curve method
    iv. Geometric correction method
2. RP – HPLC method
3. HPTLC method

5.1.1 UV SPECTROPHOTOSCOPIC METHODS:

The Metoprolol succinate and Telmisartan raw materials were identified by IR spectras shown in Figure 1 and 2. The solubility of Metoprolol succinate and Telmisartan was determined by Schefter and Higuchi method and as per Indian Pharmacopoeia. The polar and non-polar solvents were employed to dissolve the drugs. The solubility profile of Metoprolol succinate and Telmisartan was shown in Table 1 and 2. The Melting points were determined and shown in Table 3.

5. 1.1.1 SIMULTANEOUS EQUATION METHOD

The sample solutions of 10 μg mL^{-1} of metoprolol succinate and telmisartan in 0.1 M HCl prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using 0.1 M HCl as blank. The individual and overlaid spectrum of metoprolol succinate and telmisartan were recorded as shown in Figure 3 to 7. From the spectrum, 275 nm was chosen as the λ_{max} of metoprolol succinate and 228.5 nm was chosen as the λ_{max} of telmisartan. The two wave lengths were applied for Simultaneous estimation of metoprolol succinate and telmisartan.

Aliquots of metoprolol succinate and telmisartan in 0.1 M HCl was prepared in the concentration range of 2-10 μg mL^{-1} and 3.2-16μg mL^{-1}. The absorbances of solutions were measured at selected wavelengths for metoprolol succinate and telmisartan respectively. The calibration curve was plotted using concentration against absorbance. The calibration curve was plotted and shown in Figure 8 to 11. The procedure was repeated six times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for all the two drugs was found to be around 0.999. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The optical characteristics of two drugs at their selected wavelengths were shown in Table 4&5.

For Quantification, the concentration of solution containing 4 μg mL^{-1} of metoprolol succinate and 6.4 μg mL^{-1} telmisartan was prepared and measured at their respective wavelengths. The percentage purity of tablets was found to be 100.76 ± 1.2076 and 100.78 ± 0.1649 for metoprolol succinate and telmisartan respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 1.1986 and 0.1637 for metoprolol succinate and telmisartan, respectively. The low % RSD value indicates that the method has good precision. The results of analysis are shown in Table 6.

Further, the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of the formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be 0.8852 and 0.8663 for metoprolol succinate, 0.3010 and 0.3011 for telmisartan. The results of analysis are shown in Table 7. The results showed that the precision of the method was confirmed.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts and different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value of 80%, 100%, 120% by analyst 1 and analyst 2 were found to be 0.6080, 0.4330, 0.6392 and 0.1698, 0.9876, 0.3637 for metoprolol succinate and 0.1142, 1.0897, 0. 1309 and 0.1085, 0.6206, 0.2777 for telmisartan, respectively. The low % RSD values indicate that the developed method was more rugged. The results were shown in Table 8.

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulations, a known quantity of metoprolol succinate and telmisartan raw material solutions were added at three different concentrations. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.91 ± 0.3579 for metoprolol succinate and 101.28 ± 0.2419 for telmisartan. The average % RSD values of metoprolol succinate and telmisartan were found to be 0.3582 and 0.2392, respectively. The low % RSD value for drugs indicates that this method is very accurate. The recovery data is shown in Table 9.

5.1.1.2 AREA UNDER THE CURVE METHOD

The solutions containing 10 μg mL^{-1} of metoprolol succinate and telmisartan in 0.1 M HCl were prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using 0.1M HCl as blank. From the spectrum, 227.5-214 nm was chosen as the area range for metoprolol succinate and 303-278.5 nm was chosen as the area range for telmisartan. This two wave length ranges were used for the estimation of metoprolol succinate and telmisartan by area under the curve method.

Aliquots of Metoprolol succinate and Telmisartan in 0.1 M HCl was prepared in the concentration range of 2-10 μg mL^{-1} and 3.2-16μg mL^{-1}. The area of the solutions was measured at selected wavelengths for metoprolol succinate and telmisartan respectively. The calibration curve was plotted using area against concentration. The calibration graphs were plotted and are shown in Figure 12 to 15.

The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for both the drugs was found to be around 0.999. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The optical characteristics of two drugs at their selective wavelengths are shown in Table 10&11.

The concentration of solution containing 4 $\mu g\ mL^{-1}$ of metoprolol succinate and 6.4 $\mu g\ mL^{-1}$ of telmisartan was prepared and the area of the solutions was measured at their respective wavelength ranges. The percentage purity of tablets was found to be 99.71 ± 0.6881 and 99.82 ± 0.3045 for metoprolol succinate and telmisartan, respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 0.6901 and 0.3051 for metoprolol succinate and telmisartan, respectively. The results of analysis were shown in Table 12. The low % RSD value indicates that the method was highly precise.

. Further, the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be 1.4717 and 1.4801 for metoprolol succinate, 0.0301 and 0.0301 for telmisartan. The results of analysis are shown in Table 13. The results revealed that the method was highly precise.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value 80%, 100%, 120% by analyst 1 and analyst 2 was found to be 0.3575, 1.4515, 0.8402 and 0.4027, 0.4276, 0.2019 for metoprolol succinate and 0.0348, 0.4640, 0.1995 and 0.0.6038, 0.0870, 0.7029 for telmisartan, respectively. The low % RSD value indicates that the developed method was more rugged. The results were shown in Table 14.

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulations, a known quantity of metoprolol succinate and telmisartan raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 101.34 ± 1.4893 for metoprolol succinate and 99.33 ± 0.2205 for telmisartan. The average %RSD values of metoprolol succinate and telmisartan was found to be 1.4697 and 0.2220, respectively. The low % RSD value of drugs indicates that this method was accurate. The recovery data is shown in Table 15.

5.1.1.3 DERIVATIVE SPECTROPHOTOMETRIC METHOD

The zero order spectrums were derivatised into first order derivative spectrums. The individual and overlaid first order derivative spectrum of metoprolol succinate and telmisartan was recorded as shown in Figure 16 to 18. From the spectrum, 269 nm was selected for the estimation of metoprolol succinate, which is zero crossing for telmisartan and 243 nm was selected for the estimation of telmisartan which is zero crossing for metoprolol succinate. Aliquots of metoprolol succinate and telmisartan were prepared in the concentration range of 10-50 μg mL^{-1} and 16-80μg mL^{-1}. The $\Delta A/\Delta\lambda$ value of these solutions was measured at 269 nm and 243 nm in the first order derivative spectrum for metoprolol succinate and telmisartan, respectively. The plotted calibration curves were shown in Figure 19 & 20 for metoprolol succinate and telmisartan, respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against $\Delta A/\Delta\lambda$. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for both the drugs. The correlation coefficient for all the two drugs was found to be above 0.999. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The results are shown in Table16.

For Quantification, the concentration of solution containing 20 μg mL^{-1} of metoprolol succinate and 32 μg mL^{-1} of telmisartan was prepared and measured at their respective wavelengths. The amount of six test solutions was determined. The percentage purity of granules was found to be 101.01 ± 1.3426 and 101.19 ± 1.0604 for metoprolol succinate and telmisartan, respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 1.3291 and 1.0479 for metoprolol succinate and telmisartan, respectively. The results of analysis are shown in Table-17. The low % RSD value indicates that the method has good precision.

Further, the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of tablets was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of Intraday and Inter day analysis are 0.4038 and 0.3165 for metoprolol succinate, 0.3187 and 0.2676 for telmisartan, respectively. The results of analysis are shown in Table 18. Hence the precision was confirmed.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different instruments and different sources of reagents and so on. In the present work, it was confirmed by different analysts. The % RSD value of 80%, 100%, 120% by analyst 1 and analyst 2 were found to be 1.7413, 1.4707, 1.2164 and 1.3019, 1.4707, 1.2164 for metoprolol succinate and 0.4357, 1.4797, 0.2582 and 0.1755, 0.3829, 0.1293 for telmisartan, respectively. The low % RSD value indicates that the developed method was more rugged. The results are shown in Table 19.

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulations, a known quantity of metoprolol succinate and telmisartan raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.72 ± 0.6862 for metoprolol succinate, and 100.42 ± 0.4465 for telmisartan. The average %RSD values of metoprolol succinate and telmisartan was found to be 0.6813 and 0.4446, respectively. The low % RSD values of the drugs reveals that the method was more accurate. The recovery data was shown in Table 20.

5.1.1.4 GEOMETRIC CORRECTION METHOD:

In the geometrical correction, the corrected absorbance were obtained above said equation for both the drugs was solved by using simultaneous equation method which is simple, accurate, precise and used for quantitative simultaneous estimation of MET and TEL in biological sample.

The solutions containing 10 μg mL^{-1} of metoprolol succinate and telmisartan in 0.1 M HCl were prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using 0.1 M HCl as blank. From the spectrum, 217nm, 225nm, 232 nm was chosen as the wavelengths for Metoprolol succinate and 267nm, 275nm, 283nm was chosen as the wavelengths for Telmisartan. This six wave length ranges were used for the estimation of metoprolol succinate and telmisartan by geometric correction method shown in Figure- 21 to 23.

Aliquots of metoprolol succinate and telmisartan in 0.1 M HCl was prepared in the concentration range of 2-10 μg mL^{-1} and 3.2-16μg mL^{-1}. The absorbance of the solutions was measured at 217nm, 225nm, 232nm for metoprolol succinate. The calibration curve was plotted using area against concentration. The calibration graph at 217nm, 225nm, 232nm was shown in Figure 24 to 27. The area of telmisartan solutions were measured at 267nm, 275nm, 283 nm. The calibration graphs were plotted and are shown in Figure 25. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, Sandell’s sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for both the drugs was found to be around 0.99. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The optical characteristics of two drugs at their selective wavelengths are shown in Table 21&22.

The concentration of solution containing 4 $\mu g\ mL^{-1}$ of metoprolol succinate and 6.4 $\mu g\ mL^{-1}$ of telmisartan were prepared and the absorbance of the solutions was measured at their respective wavelength ranges. The percentage purity of Tablets was found to be 100.00 ± 0.3019 and 100.17 ± 0.3058 for metoprolol succinate and telmisartan, respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 0.3018 and 0.3052 for metoprolol succinate and telmisartan, respectively. The results of analysis were shown in Table 23. The low % RSD value indicates that the method was highly precise. .

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulations, a known quantity of metoprolol succinate and telmisartan raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.88 ± 0.5521 for metoprolol succinate and 100.14 ± 0.2145 for telmisartan. The average %RSD values of metoprolol succinate and telmisartan was found to be 0.5528 and 0.2142, respectively. The low % RSD value of drugs indicates that this method was accurate. The recovery data is shown in Table 24.

5.1.2 RP – HPLC METHOD

A simple, rapid, accurate and precise method for the estimation of metoprolol succinate and telmisartan in pure and in tablet dosage form by an isocratic RP – HPLC method.

The solutions of 10 μg mL^{-1} of metoprolol succinate and telmisartan in mobile phase Acetonitrile: Phosphate buffer pH 3 (25:75 v/v) were prepared and the solutions were scanned in the range of 200 – 400 nm. It was found that all two drugs have marked absorbance at 230 nm and can be effectively used for estimation of two drugs without interference. The selection of detection wavelength was shown in Figure 28. Therefore 230 nm was selected as detection wavelength for estimation of two drugs by RP – HPLC method with an isocratic elution technique and it was found that two drugs are stable for approximately 24 hours.

The optimization was done by changing the composition of mobile phase, ratio and pH. After calculating all system suitability parameters, Acetonitrile: Phosphate buffer pH 3 with the ratio of 25:75 v/v at a flow rate of 1.0 ml min^{-1} was selected and the optimized chromatogram was shown in Figure 23. The retention time of metoprolol succinate and telmisartan were found to be 2.45 and 6.99, respectively. The retention time between two drugs indicates that the drugs were separated with better resolution of 4.5 between metoprolol succinate and telmisartan. The system suitability parameters for the optimized chromatogram are shown in Table 25.

With the optimized chromatographic conditions, stock solutions containing metoprolol succinate and telmisartan 10 μg mL^{-1} were prepared in mobile phase. The stock solution was further diluted to concentration range of 30-70 μg mL^{-1} of metoprolol, 48-112 μg mL^{-1} of telmisartan. 20 μL of each solution was injected and recorded the chromatograms at 230 nm. The chromatograms were shown in Figure 29 to 33. The calibration curve was plotted using concentration against peak area. The procedure was repeated for six times. The correlation co-efficient was found to be around 0.999 for both the drugs. The calibration graph of Metoprolol succinate and Telmisartan was shown in Figure 34 and 35, respectively. The optical characteristics of metoprolol succinate and telmisartan were shown in Table 26.

The six test concentration containing 50 μg mL^{-1} metoprolol succinate and 80 μg mL^{-1} telmisartan was prepared from formulation. 20 μL of each solution was injected and chromatograms were recorded. The percentage purity was found to be 100.02 ± 0.4736 and 99.33 ± 0.3189 for metoprolol succinate and telmisartan, respectively. The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms were shown in Figure 36 to 41. The percentage RSD was found to be 0.4734 and 0.3211 for metoprolol succinate and telmisartan, respectively. It indicates that the method has good precision. The data is shown in Table 27.

The accuracy of the method was performed by recovery studies. To the 100% preanalyzed formulations, a known quantity of metoprolol succinate and telmisartan raw material solutions were added at three different concentration levels. The chromatograms were recorded as shown in the Figure 42 to 44. The percentage recovery was found to be in the range between 101.92 and 103.53% for metoprolol succinate and 98.93 and 100.05% for telmisartan. The average %RSD values of metoprolol succinate and telmisartan was found to be 0.8933 and 0.5763, respectively. The low % RSD values for recovery indicated that the method was accurate. The values are given in the Table 28. The high percentage recovery revealed that no interference produced due to the excipients used in tablets. Therefore, the developed method was found to be more accurate. All the above parameters with the ease of operation ensure that the projected methods could be applied for the routine analysis of metoprolol succinate and telmisartan in pure form and in tablet dosage forms.

5.1.3 HIGH PERFORMANCE THIN LAYER LIGUID CHROMATOGRAPHY

A simple, rapid, accurate and precise method for the estimation of Metoprolol succinate and Telmisartan in pure and in tablet dosage form by HPTLC method.

The solutions of 10 μg mL^{-1} of metoprolol succinate and telmisartan in mobile phase Chloroform: Methanol: Formic acid in the ratio of (85: 15: 2.5%v/v) were prepared and the solutions were scanned in the range of 200 – 400 nm. It was found that all two drugs have marked absorbance at 233 nm and can be effectively used for estimation of two drugs without interference. The selection of detection wavelength was shown in Figure 45. Therefore 233 nm was selected as detection wavelength for estimation of two drugs by HPTLC method and it was found that two drugs are stable for approximately 3 hours.

Various mobile phase systems were prepared and trials were made with pre-coated plates to determine appropriate chromatographic conditions. The mobile phase finalized for HPTLC analysis was Chloroform: Methanol: Formic acid in the ratio of (85: 15: 2.5%v/v) and the chromatogram was developed. The R_f value of metoprolol succinate and telmisartan were found to be 0.45 and 0.58, respectively. The scanning of the developed plates shows a good peak shape.

The Linearity of HPTLC method developed for metoprolol succinate and telmisartan was evaluated by injecting the standard drug solutions of different concentration ranging from 1 – 5 µg mL^{-1} and 1.6 -8 µg mL^{-1} chromatogram were shown in Figure 46 to 50 and the peak areas obtained spectrum of metoprolol succinate and telmisartan in all the tracks are presented in Figure 51. The calibration curve was plotted using concentration against peak area. The procedure was repeated for six times. The correlation co-efficient was found to be around 0.999 for both the drugs. The calibration graph of metoprolol succinate and telmisartan was shown in Figure 52 and 53, respectively.

The various optical parameters like Regression equation, Slope, Intercept, LOD, LOQ, Sandell's sensitivity, standard error were calculated and the results are presented in Table 29.

The Assay determined the contents of metoprolol succinate and telmisartan formulation METOSARTAN-25 were found to be 100.89 ± 0.4755 and 100.55 ± 0.5606. The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms were shown in Figure 54 to 59. The Assay results presented in the Table 30.

The Accuracy and Precision was determined for metoprolol succinate and telmisartan by fortifying sample with standard drug substance at overall recovery determined, the contents of METOSARTAN-25 the percentage recovery was found to be 100.61% for metoprolol succinate, and 100.81% for telmisartan. The chromatograms were recorded as shown in the Figure 60 to 62. The % RSD values were found to be 0.9053 and 0.7429 for metoprolol succinate and telmisartan respectively. The low % RSD value for both drugs indicates that this method is very accurate.

The proposed HPTLC method for estimation of metoprolol succinate and telmisartan in tablet dosage form is simple, accurate and rapid. The statistical parameters in the method validation studies for Precision and Accuracy with no interference, which was proved by recovery studies, the data presented in the Table 31. Difference between shorter and longer wavelength of metoprolol succinate and telmisartan were recorded as shown in Figure 63.

6. SUMMARY

AND

CONCLUSION

SUMMARY AND CONCLUSION

Metoprolol succinate and Telmisartan drugs are used for the treatment of hypertension. Simple, rapid, precise and accurate UV Spectroscopic method, RP – HPLC AND HPTLC method were developed and validated for the estimation of metoprolol succinate and telmisartan in pure, pharmaceutical dosage form and biological sample.

6.1 UV Spectroscopic method

From the solubility data, 0.1 M HCl was selected as the solvent. Metoprolol succinate and telmisartan (10 $\mu g\ mL^{-1}$) solution were prepared separately and scanned in UV region. From overlaid spectrum, 275 nm and 228.5 nm were selected for detection wavelength for Simultaneous Equation method. The percentage purity present in tablets was found to be 100.76 ± 1.2076 and 100.78 ± 0.1649 for metoprolol succinate and telmisartan respectively. The percentage recovery was found to be in the range of 99.91 ± 0.3579 for metoprolol succinate and 101.28 ± 0.2419 for telmisartan.

In area under the curve method, the wavelength ranges 227.5-214 nm and 303-278.5 nm were selected for the estimation of metoprolol succinate and telmisartan respectively. The percentage label claim present in tablets was found to be 99.71 ± 0.6881 and 99.82 ± 0.3045 for metoprolol succinate and telmisartan, respectively. The percentage recovery was found to be in the range of 101.34 ± 1.4893 for metoprolol succinate and 99.33 ± 0.2205 for telmisartan

The same spectrum were derivatised and from overlaid spectrum 269 nm selected for detection of metoprolol succinate where telmisartan shows zero crossing and also 243 nm selected for detection of telmisartan where metoprolol succinate show zero crossing. The percentage label claim present in tablets was found to be 101.01 ± 1.3426 and 101.19 ± 1.0604 for metoprolol succinate and telmisartan, respectively. The percentage recovery was found to be in the range of 100.72 ± 0.6862 for metoprolol succinate, and 100.42 ± 0.4465 for telmisartan..

In the geometrical correction, the corrected absorbance was obtained above said equation for both the drugs were solved by using simultaneous equation method. The wavelength ranges 217 nm, 225 nm, 232 nm and 267 nm, 275 nm, 283 nm were selected for the estimation of metoprolol succinate and telmisartan respectively. The percentage label claim present in tablets was found to be 100.00 ± 0.3019 and 100.17 ± 0.3058 for metoprolol succinate and telmisartan, respectively. The percentage recovery was found to be in the range of 99.88 ± 0.5521 for metoprolol succinate and 100.14 ± 0.2145 for telmisartan.

6.2 RP – HPLC METHOD

In RP-HPLC method, the mobile phase used is Acetonitrile: Phosphate buffer pH 3 (25:75 v/v) with flow rate of 1 mL per minute, the retention time of metoprolol succinate and telmisartan were found to be 2.45 and 6.99, respectively at 230 nm.

The percentage purity was found to be 100.02 ± 0.4736 and 99.33 ± 0.3189 for metoprolol succinate and telmisartan respectively. The precision of the method was confirmed by repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range between 101.92 and 103.53% for metoprolol succinate and 98.93 and 100.05% for telmisartan. The low % RSD values for recovery indicated that the method was accurate.

6.3 HIGH PERFORMANCE THIN LAYER LIGUID CHROMATOGRAPHY

In HPTLC method, the mobile phase used is Chloroform: Methanol: Formic acid in the ratio of (85: 15: 2.5%v/v) and the chromatogram was developed. The R_f value of metoprolol succinate and telmisartan were found to be 0.45 and 0.58, respectively at 232 nm. The scanning of the developed plates shows a good peak shape.

The percentage purity was found to be 100.89 ± 0.4755 and 100.55 ± 0. for metoprolol succinate and telmisartan respectively. The precision of the method was confirmed by repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range between 100.61% for metoprolol succinate, and 100.81% for telmisartan. The low % RSD values for recovery indicated that the method was accurate.

Simple, rapid and accurate UV Spectroscopic (Simultaneous Equation method, First order derivative method, Area under the curve method and Geometric correction method) and an isocratic RP - HPLC and HPTLC methods showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. Hence it is suggested that the proposed UV Spectroscopic, an isocratic RP – HPLC and HPTLC methods can be effectively applied for the routine analysis of metoprolol succinate and telmisartan in bulk, biological sample and in tablet formulation and the obtained results will be presented elsewhere.

7. FIGURES

FIGURE-1

IR SPECTRUM OF METOPROLOL SUCCINATE

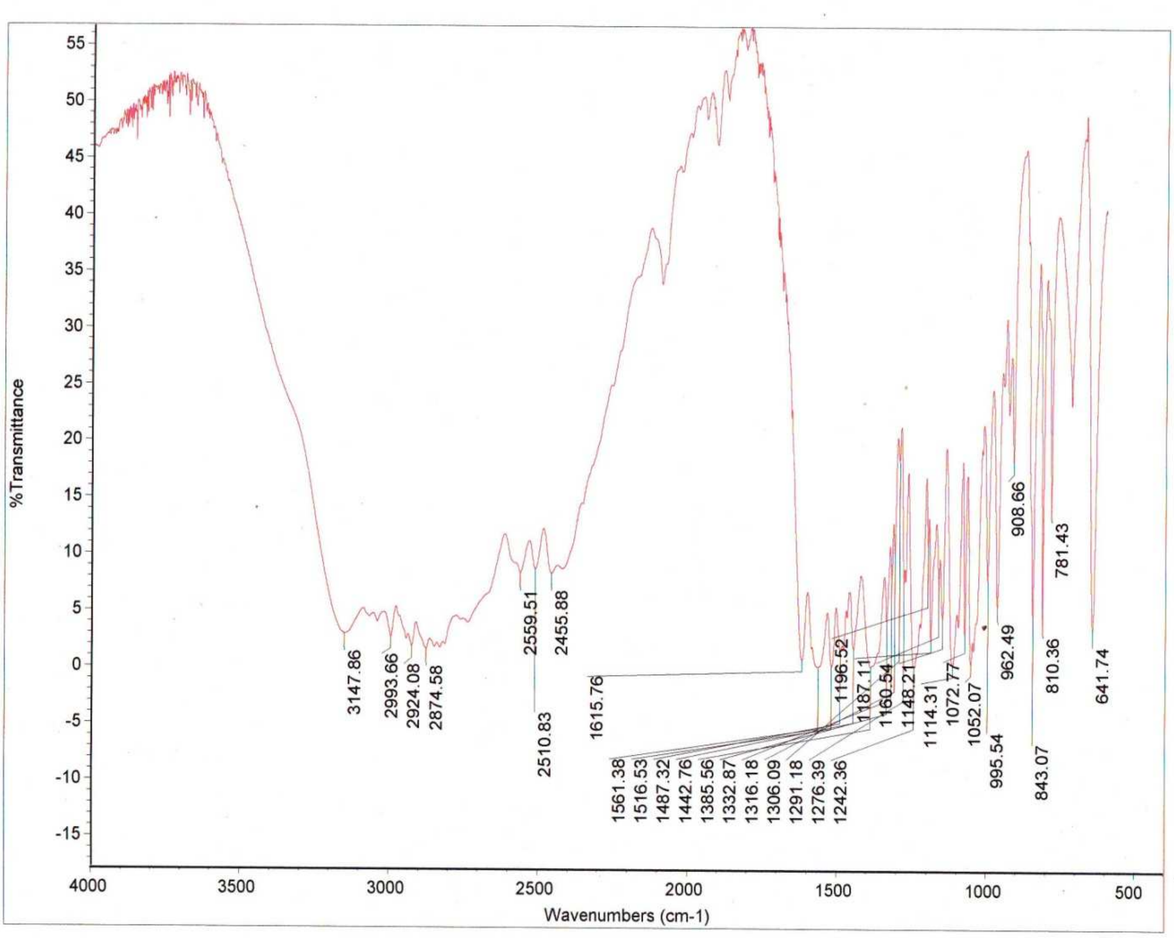


FIGURE-2

IR SPECTRUM OF TELMISARTAN

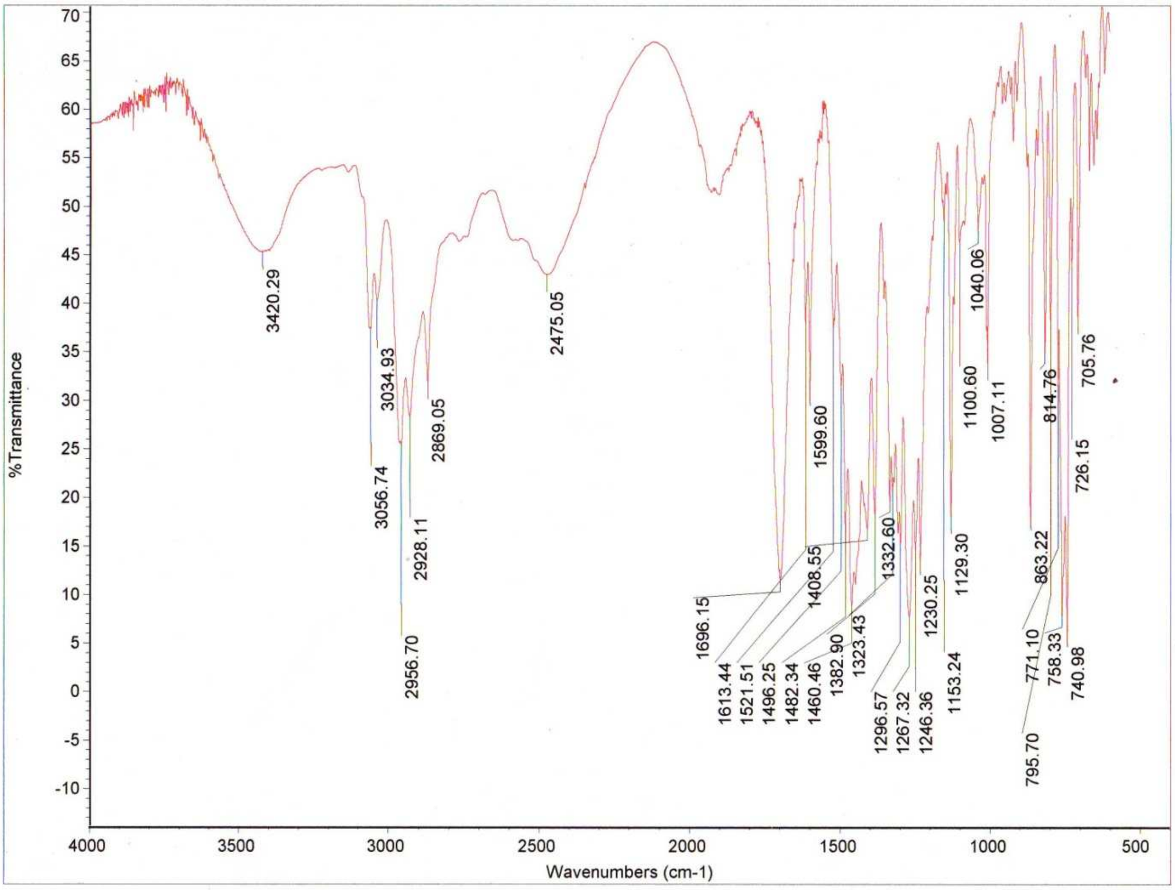


FIGURE-3

UV SPECTRUM OF METOPROLOL SUCCINATE IN 0.1M HCL AT 222 nm

(MAXIMUM ABSORPTION)

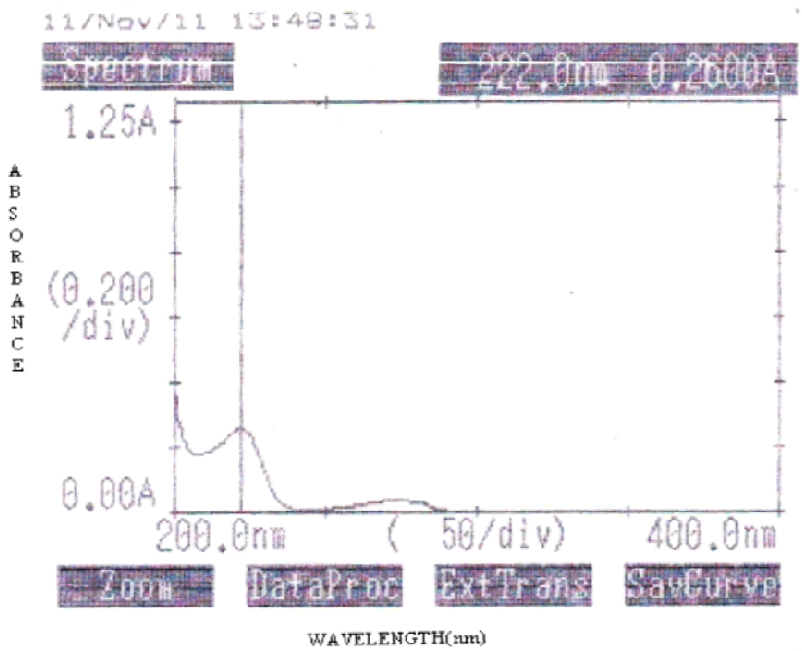


FIGURE-4

UV SPECTRUM OF TELMISARTAN IN 0.1M HCL AT 291 nm

(MAXIMUM ABSORPTION)

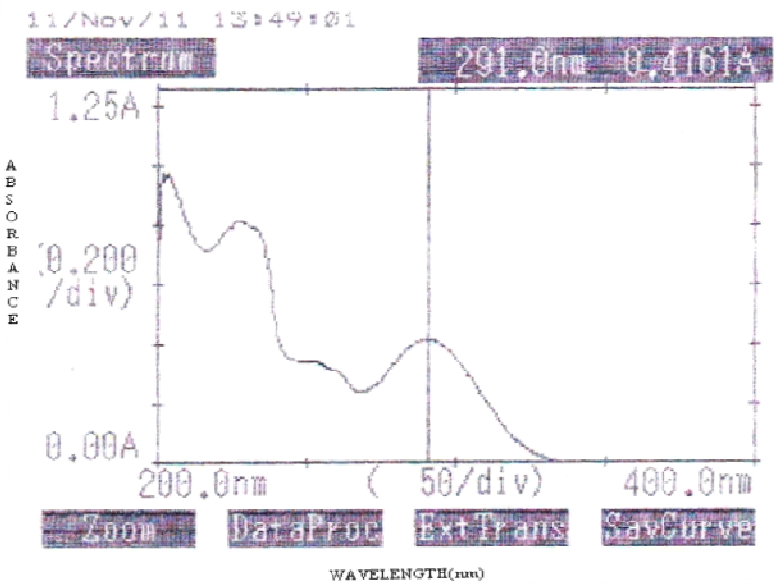


FIGURE-5

UV SPECTRUM OF METOPROLOL SUCCINATE IN 0.1M HCL AT 275 nm

(SIMLTANEOUS EQUATION METHOD)

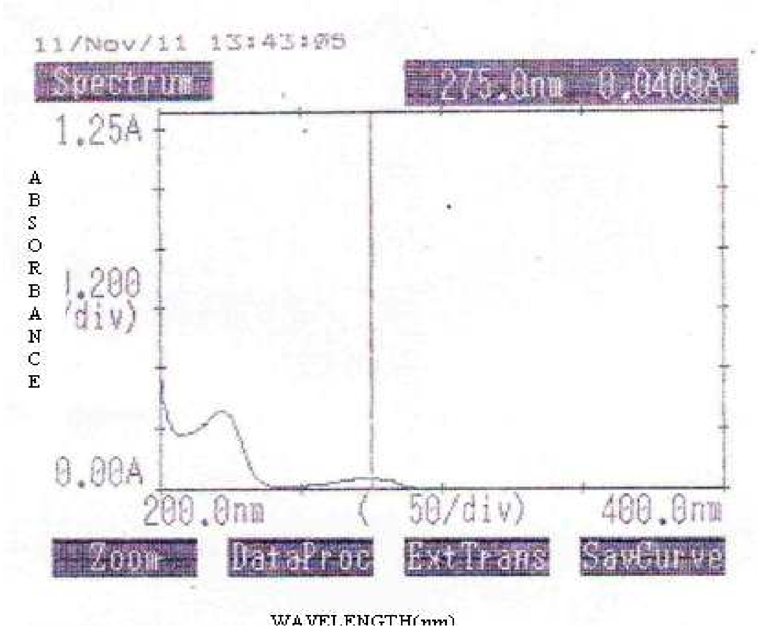


FIGURE-6

UV SPECTRUM OF TELMISARTAN IN 0.1M HCL AT 228.5 nm

(SIMLTANEOUS EQUATION METHOD)

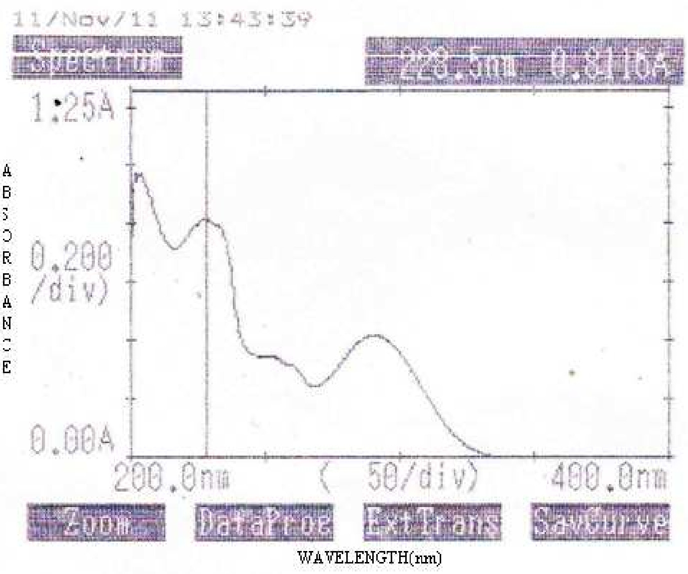


FIGURE-7

OVERLAID SPECTRA OF METOPROLOL SUCCINATE AND TELMISARTAN (SIMULTANEOUS EQUATION)

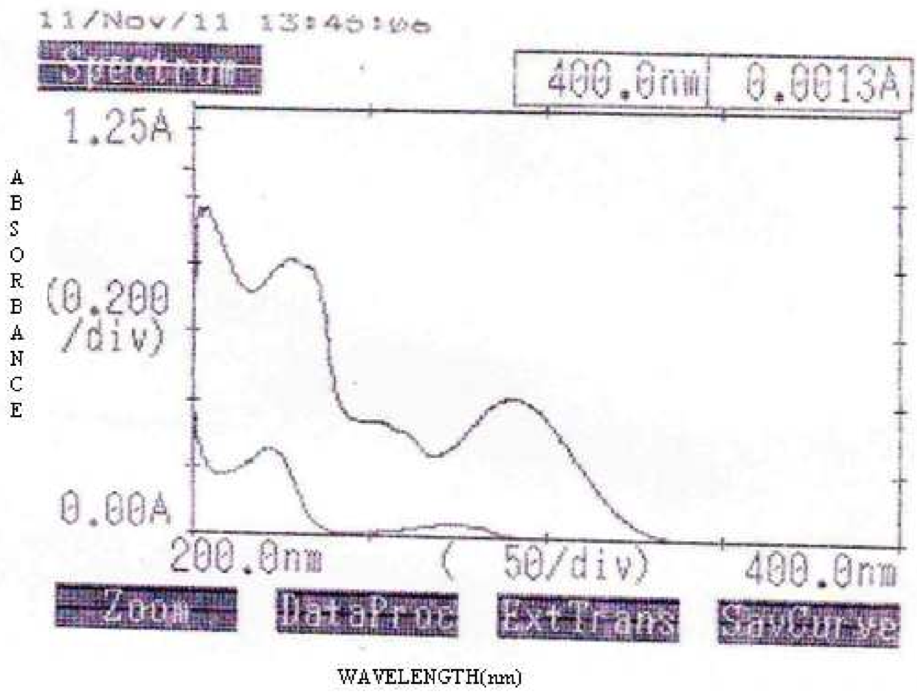


FIGURE-8

CALIBRATION CURVE FOR METOPROLOL SUCCINATE IN 0.1M HCL AT 275 nm

(SIMULTANEOUS EQUATION METHOD)

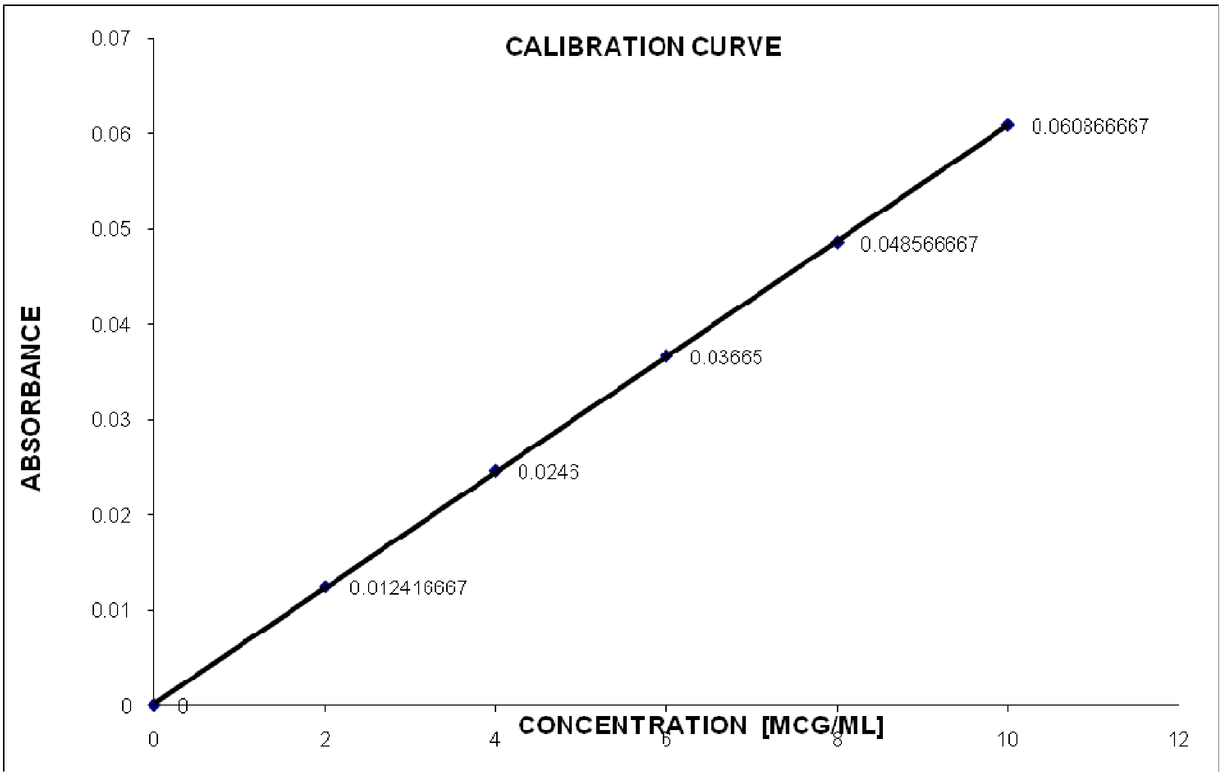


FIGURE-9

CALIBRATION CURVE FOR METOPROLOL SUCCINATE IN 0.1M HCL AT 228.5nm

(SIMULTANEOUS EQUATION METHOD)

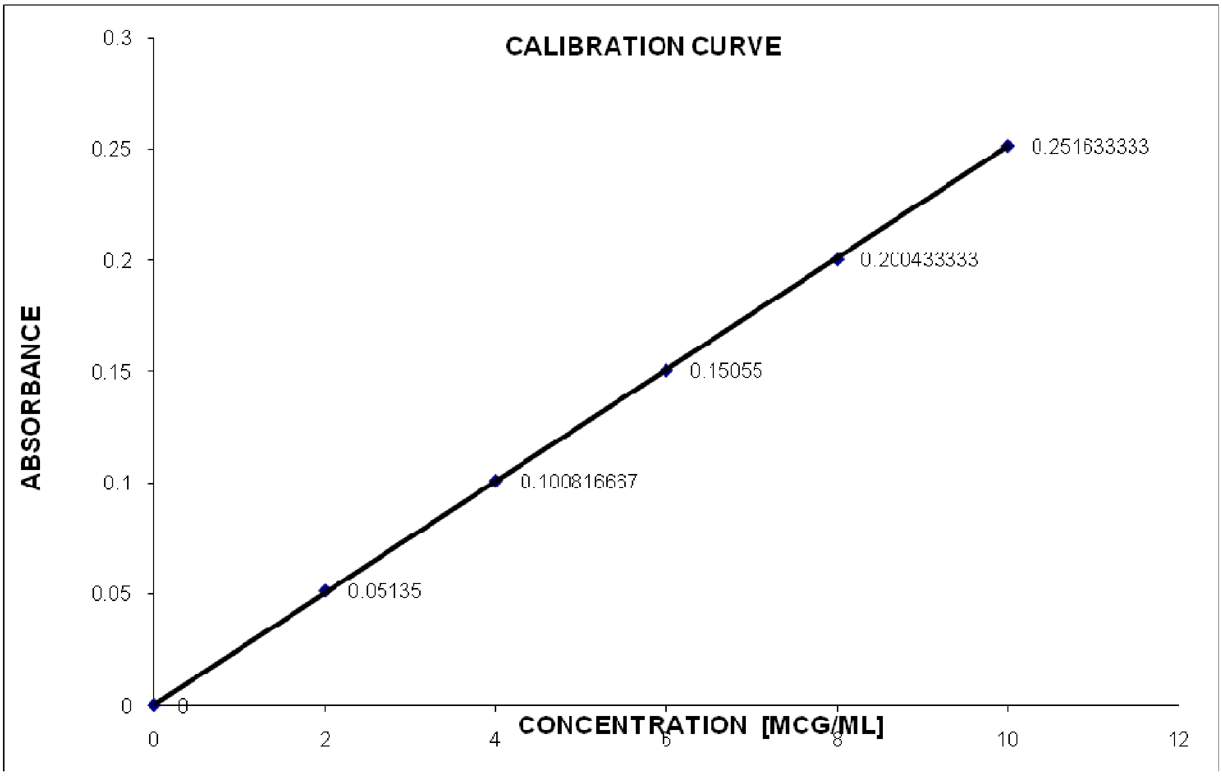


FIGURE-10

CALIBRATION CURVE FOR TELMISARTAN IN 0.1M HCL AT 228.5 nm

(SIMULTANEOUS EQUATION METHOD)

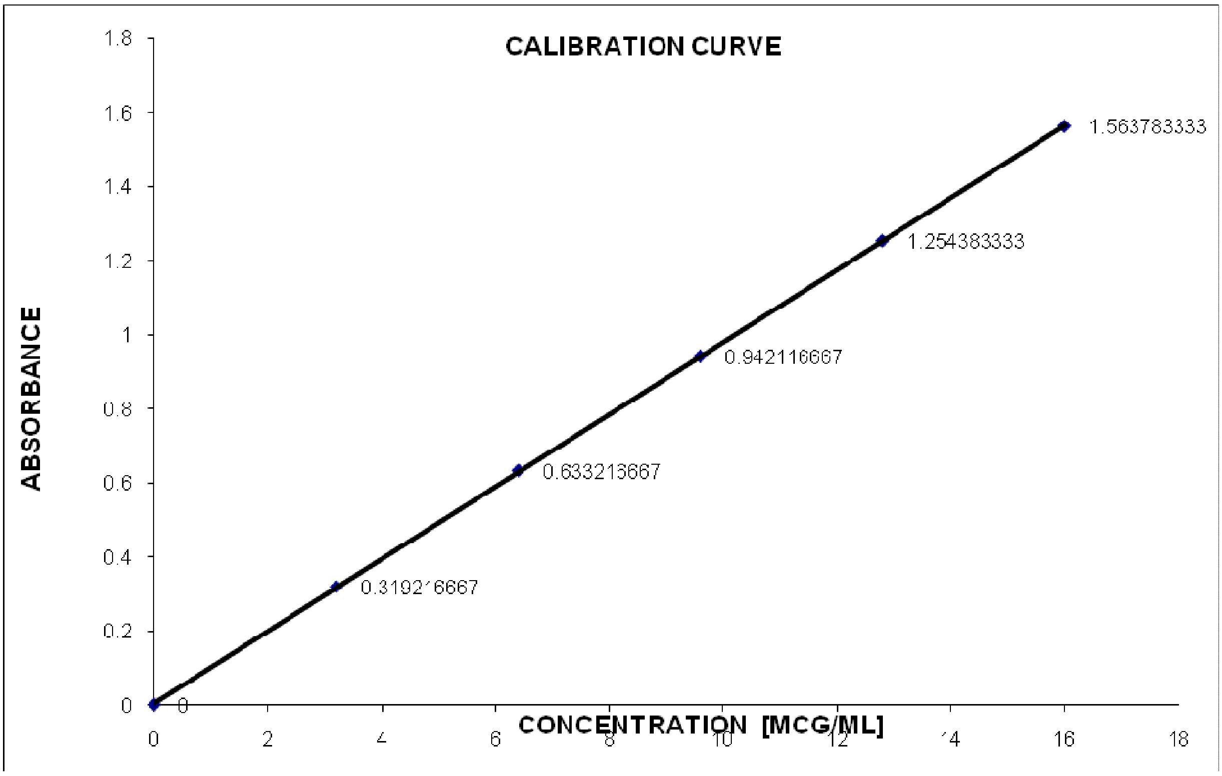


FIGURE-11

CALIBRATION CURVE FOR TELMISARTAN IN 0.1M HCL AT 275 nm

(SIMULTANEOUS EQUATION METHOD)

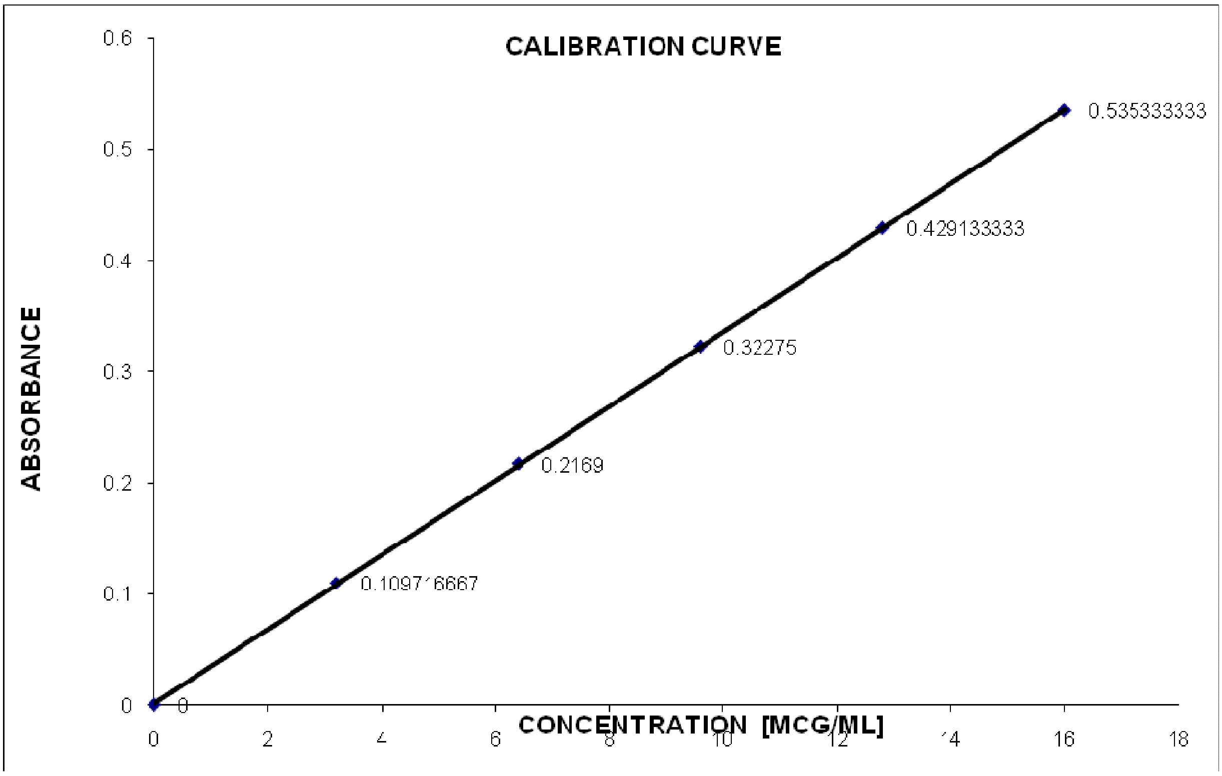


FIGURE-12

CALIBRATION CURVE FOR METOPROLOL SUCCINATE AT 227.5-214 nm

(AREA UNDER CURVEMETHOD)

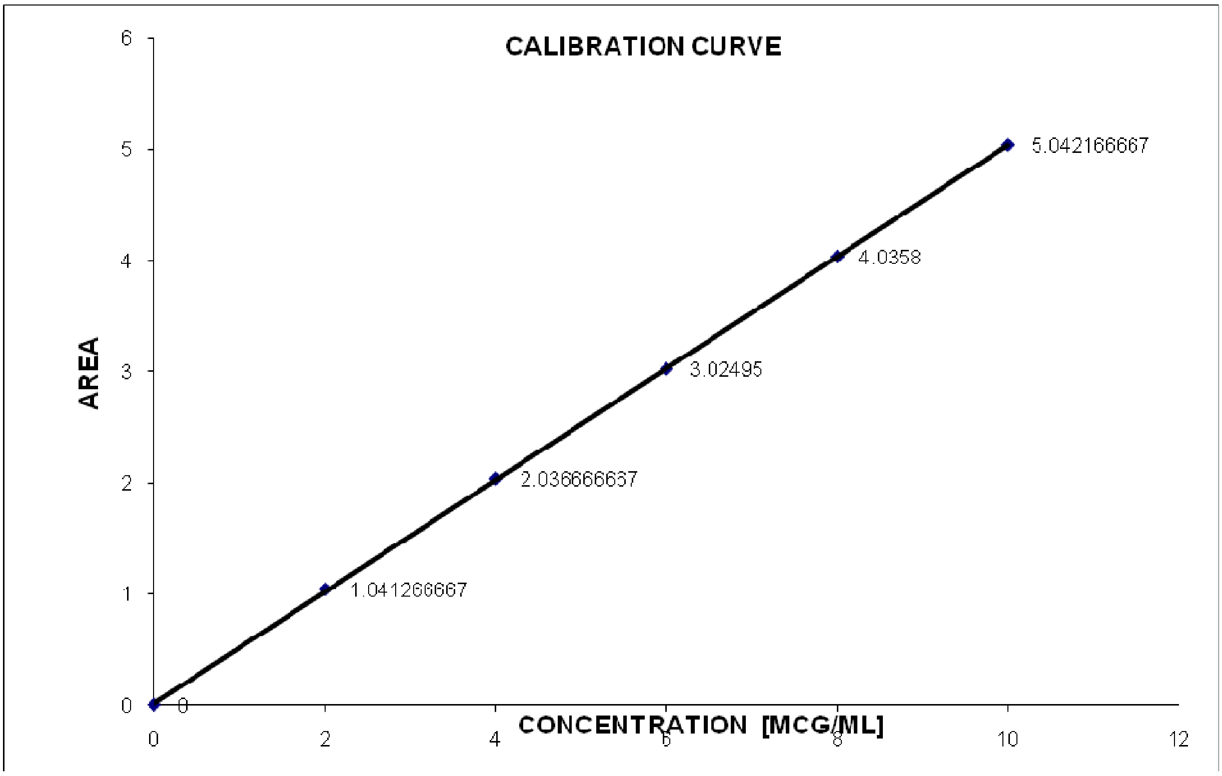


FIGURE-13

CALIBRATION CURVE FOR METOPROLOL SUCCINATE AT 303-278.5nm

(AREA UNDER CURVEMETHOD)

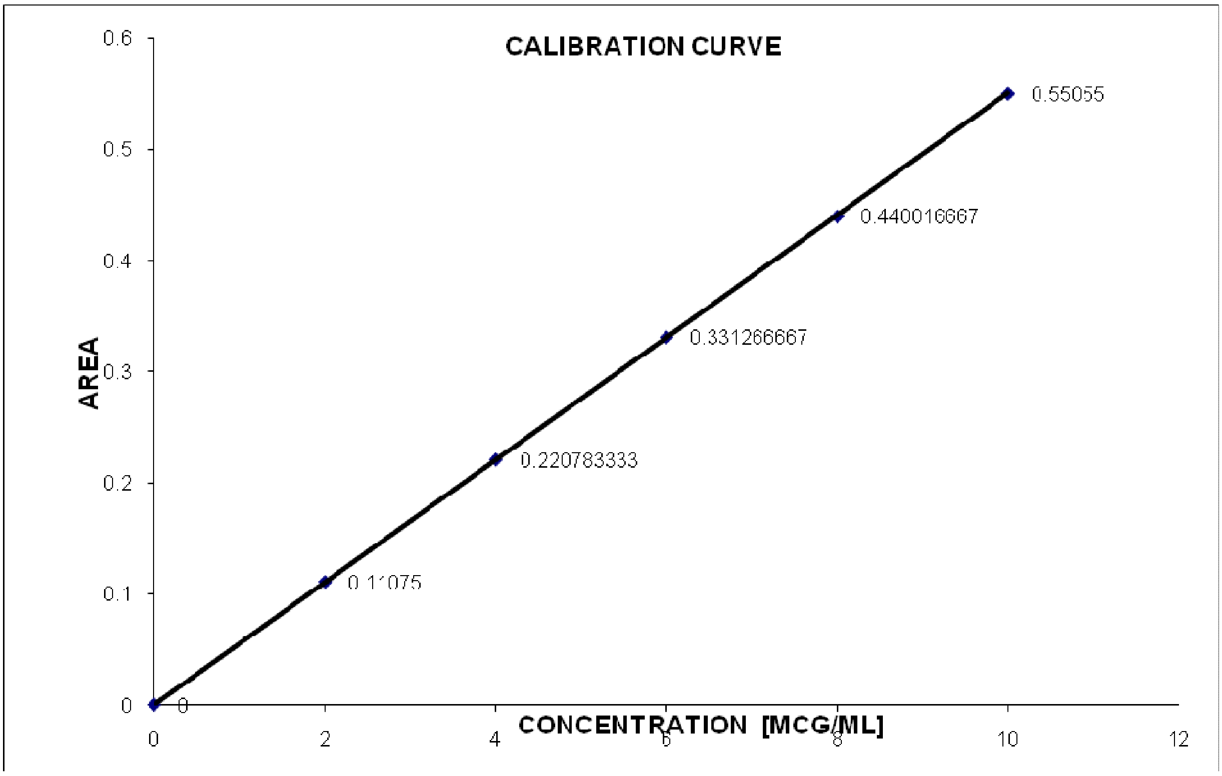


FIGURE-14

CALIBRATION CURVE FOR TELMISARTAN AT 303- 278.5 nm

(AREA UNDER METHOD)

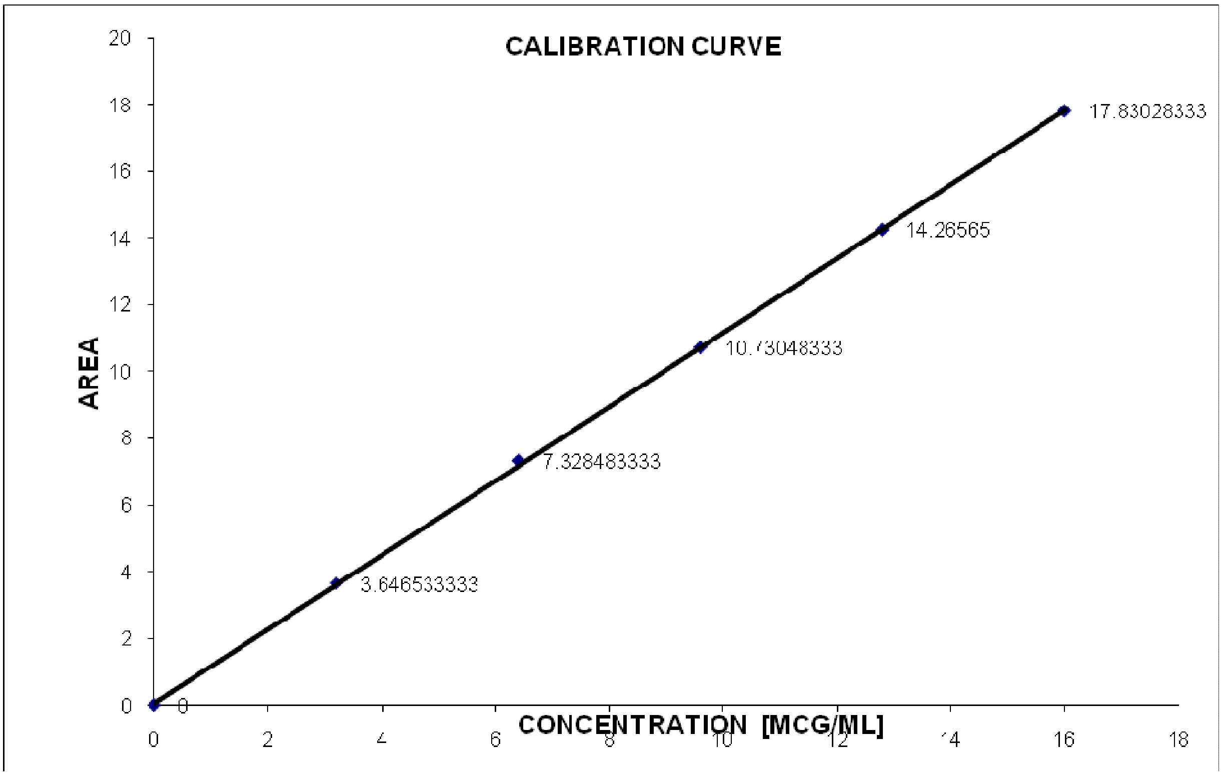


FIGURE-15

CALIBRATION CURVE FOR TELMISARTAN AT 227.5-214 nm

(AREA UNDER METHOD)

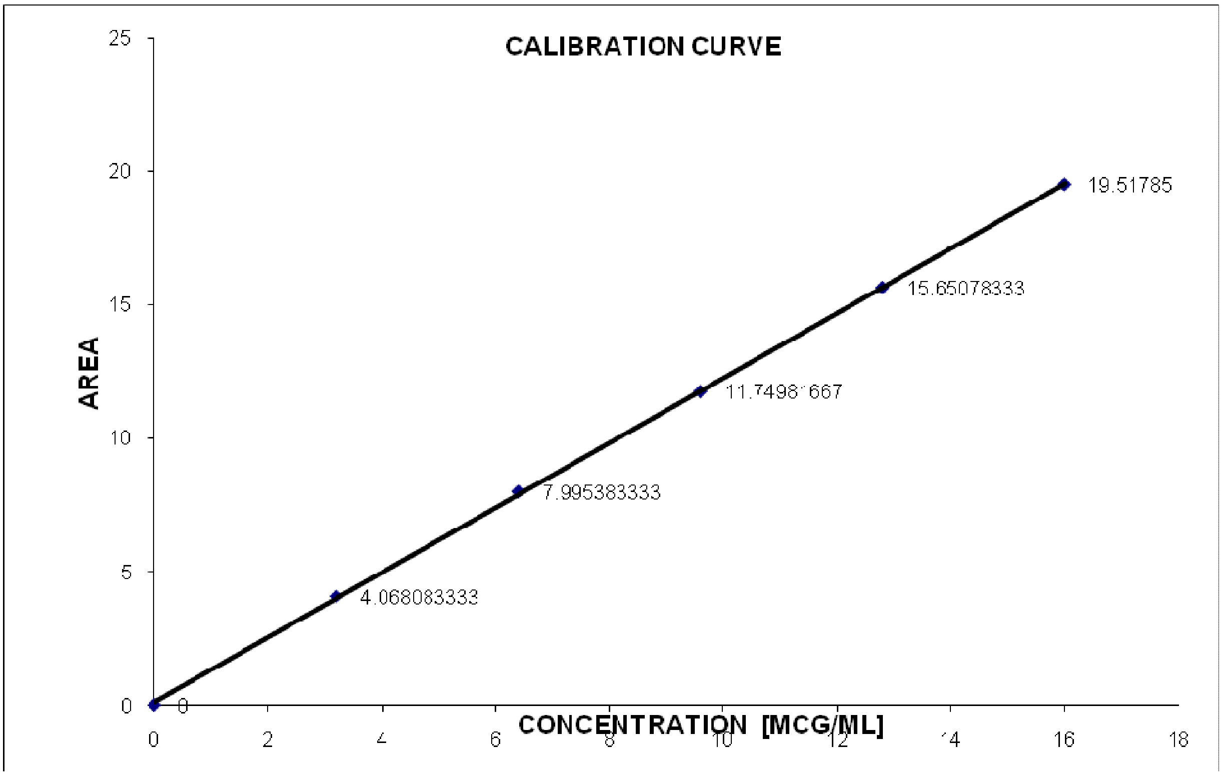


FIGURE-16

FIRST ORDER DERIVATIVE SPECTRUM OF METOPROLOL SUCCINATE

IN 0.1M HCL AT 269 nm

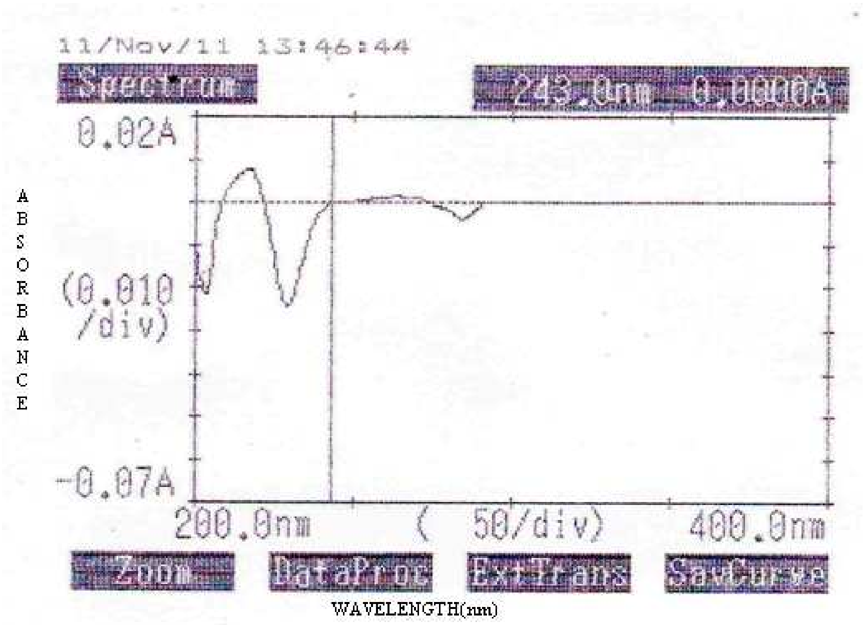


FIGURE-17

FIRST ORDER DERIVATIVE SPECTRUM OF TELMISARTAN

IN 0.1M HCL AT 243 nm

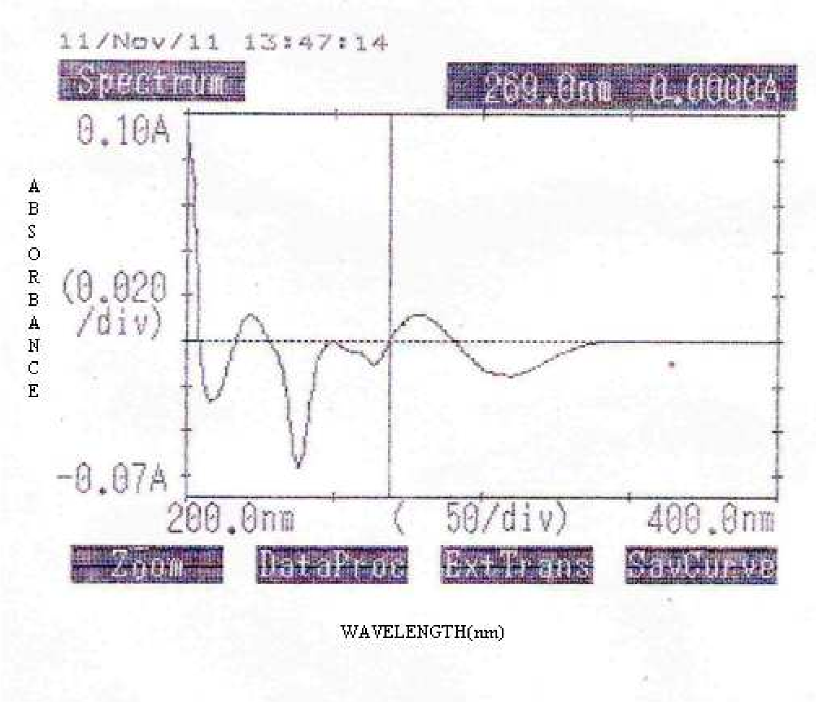


FIGURE-18

OVERLAID SPECTRUM OF FIRST ORDER DERIVATIVE OF METOPROLOLSUCCINATE AND TELMISARTAN IN 0.1M HCL

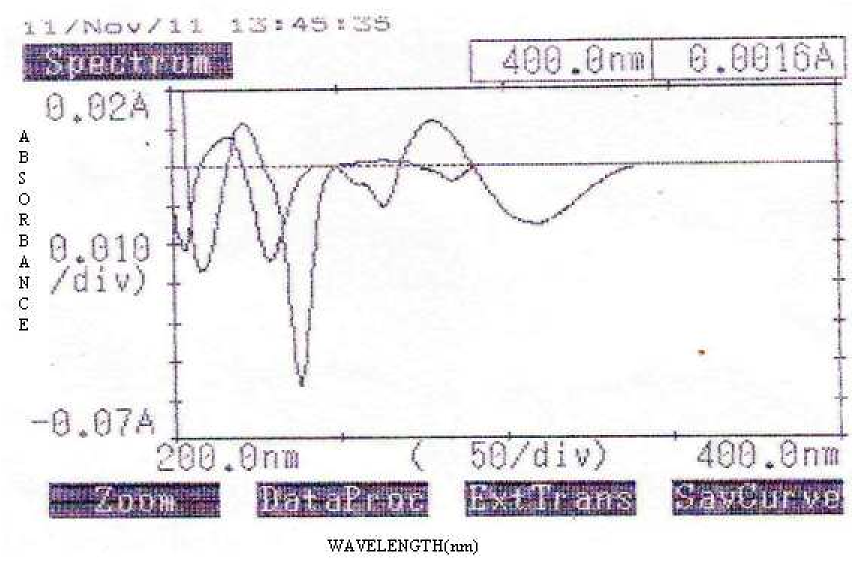


FIGURE-19

CALIBRATION CURVE FOR METOPROLOL SUCCINATE AT 269 nm

(FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD)

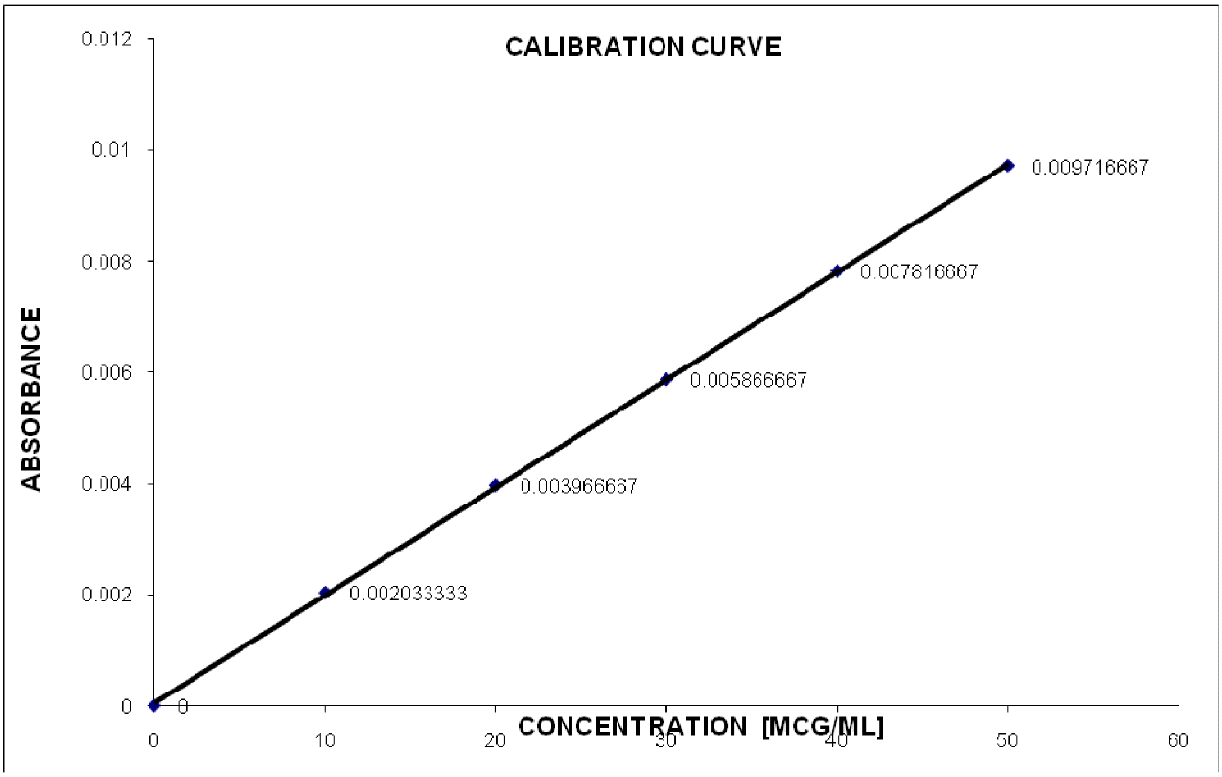


FIGURE-20

CALIBRATION CURVE FOR TELMISARTAN AT 243 nm

(FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD)

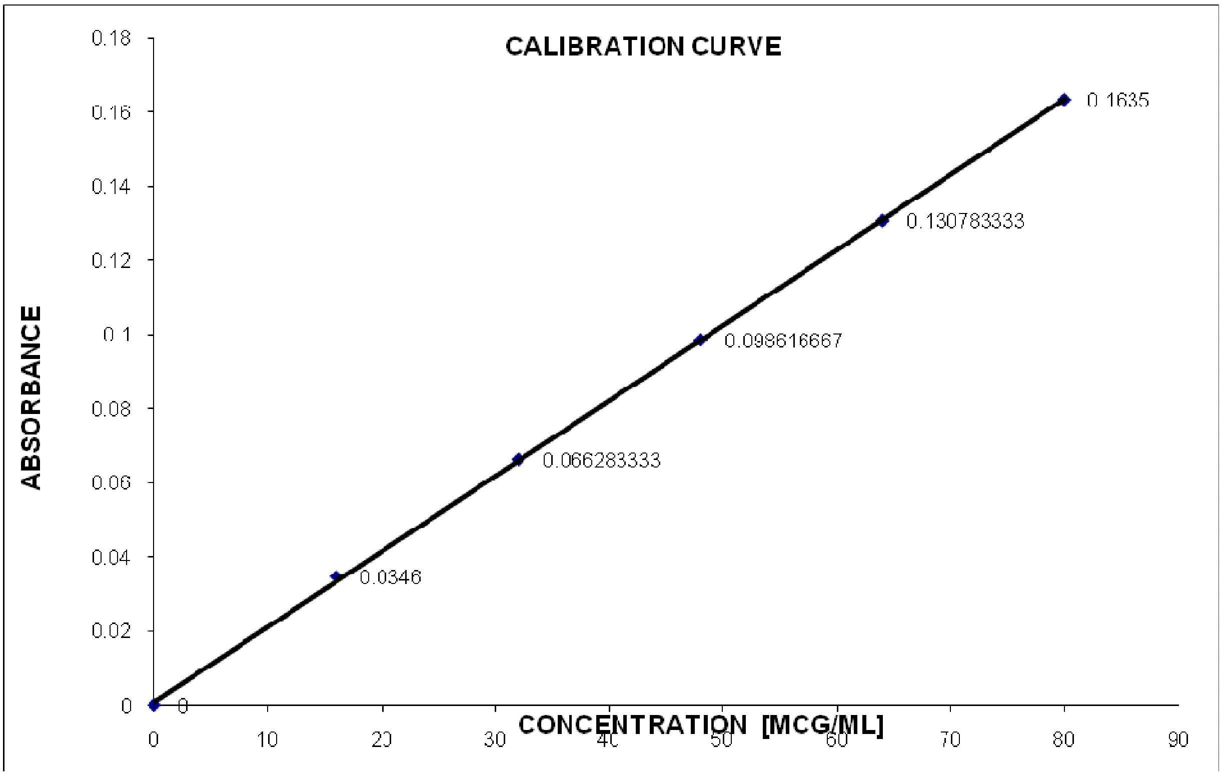


FIGURE-21

OVERLAID SPECTRUM OF GEOMETRIC CORRECTION METHOD OF METOPROLOL SUCCINATE IN 0.1M HCL WITH ARTIFICIAL URINE

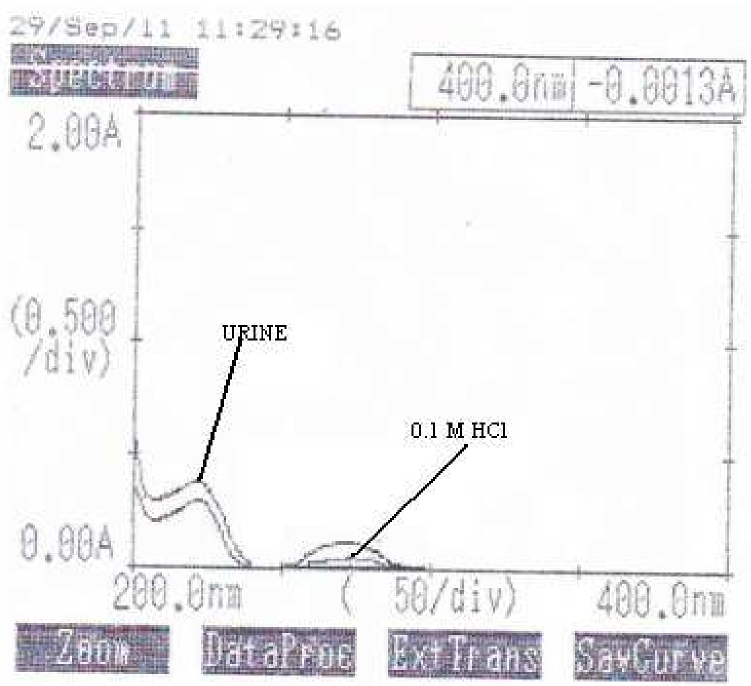


FIGURE-22

OVERLAID SPECTRUM OF GEOMETRIC CORRECTION METHOD OF TELMISARTAN IN 0.1M HCL WITH ARTIFICIAL URINE

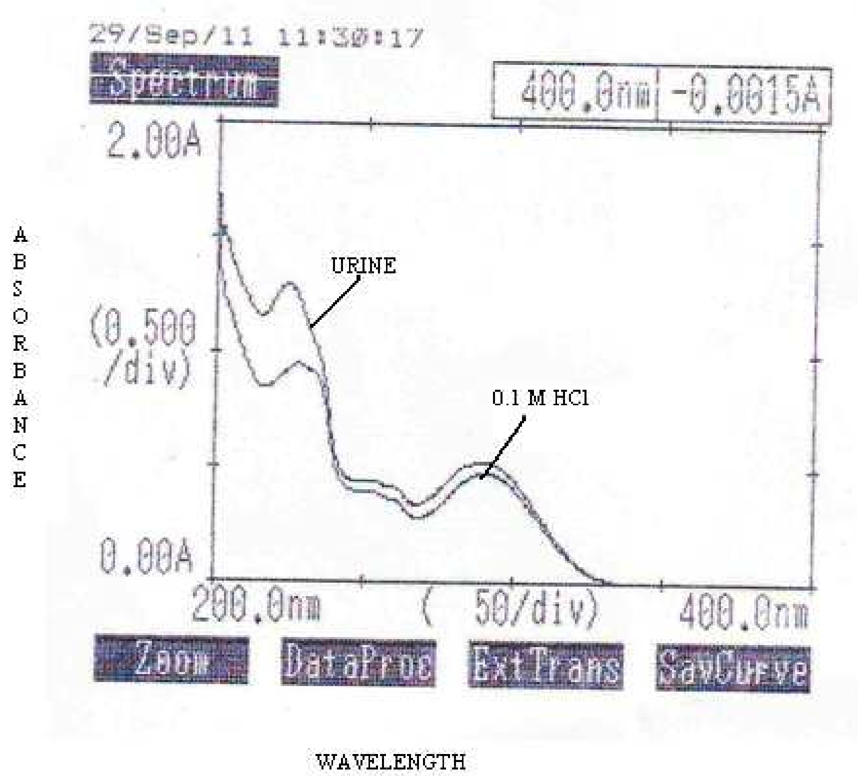


FIGURE-23

OVERLAID SPECTRUM OF GEOMETRIC CORRECTION METHOD OF METOPROLOL SUCCINATE AND TELMISARTAN IN 0.1M HCL WITH ARTIFICIAL URINE

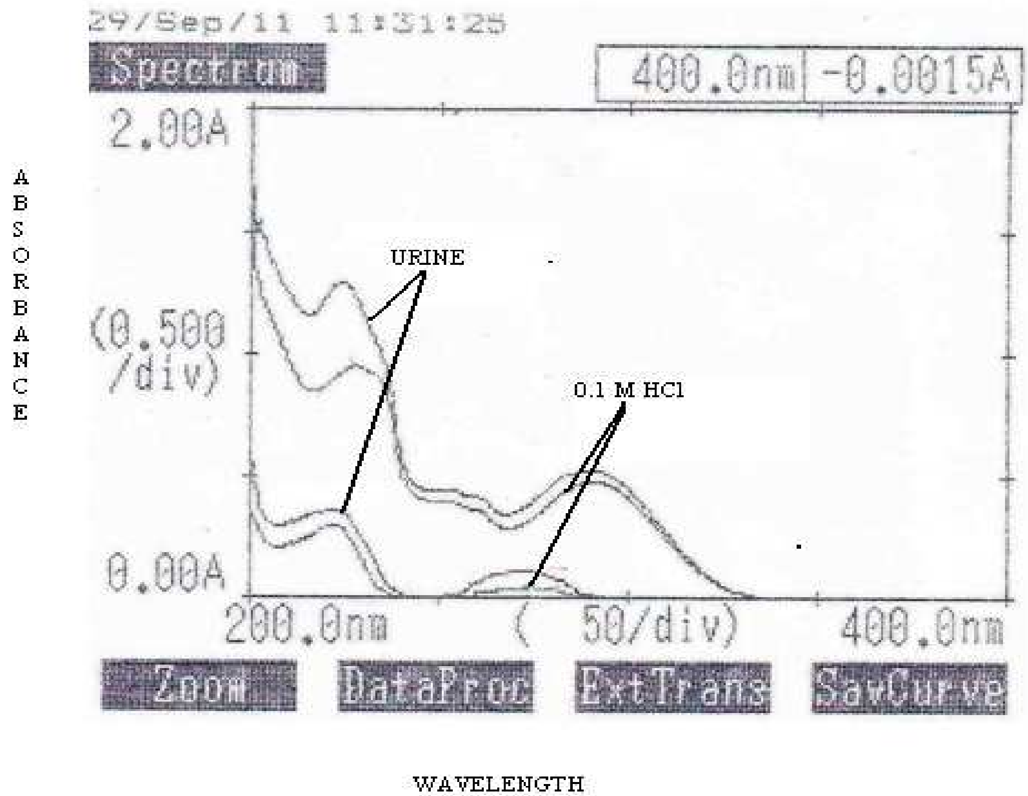


FIGURE-24

CALIBRATION CURVE FOR METOPROLOL SUCCINATE AT 217,225,232 nm

(GEOMETRIC CORRECTTION METHOD)

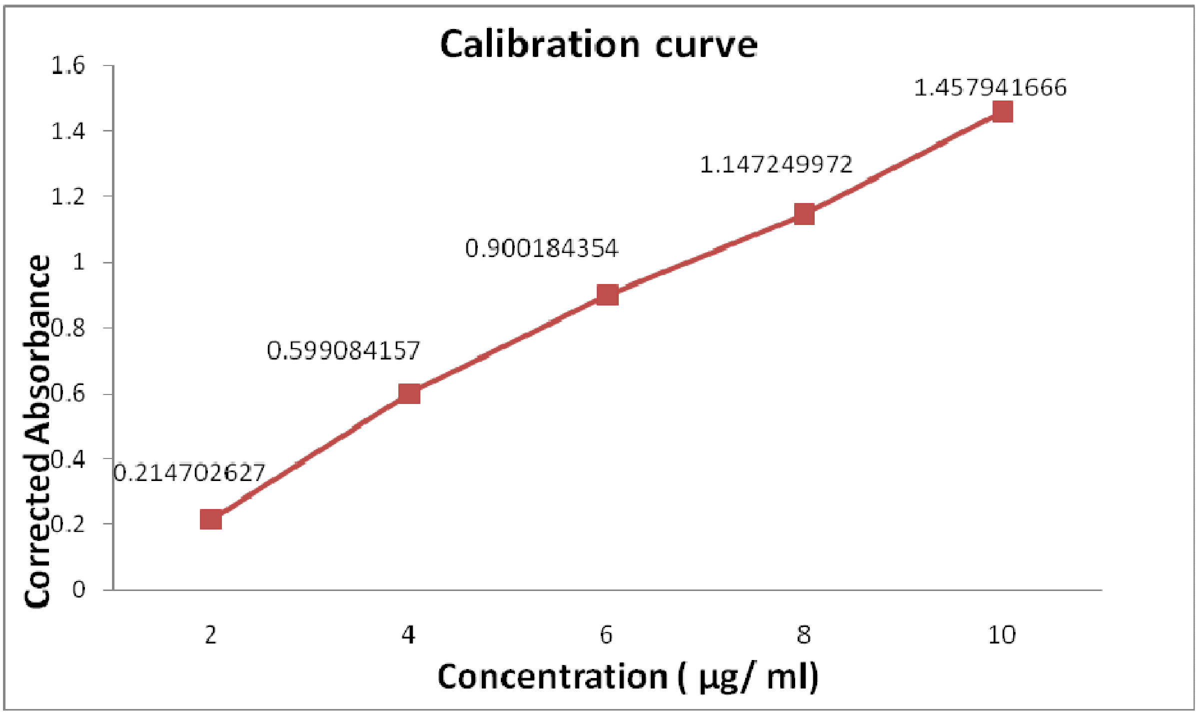


FIGURE-25

CALIBRATION CURVE FOR METOPROLOL SUCCINATE AT 267,275,283 nm

(GEOMETRIC CORRECTTION METHOD)

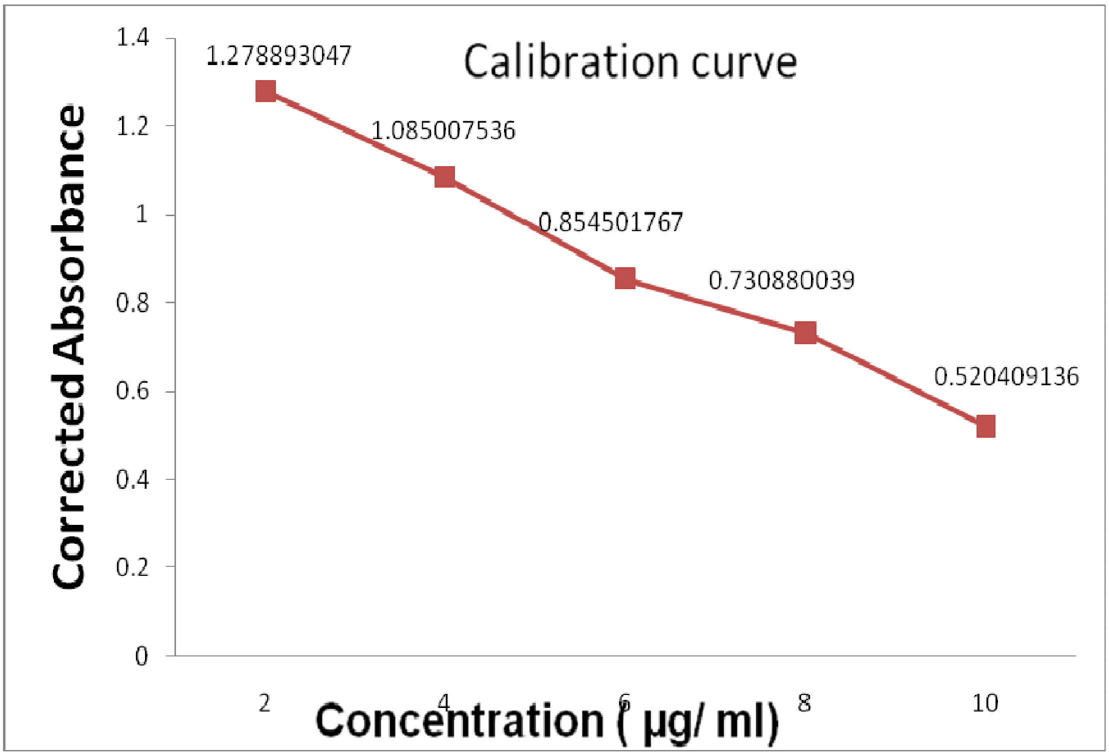


FIGURE-26

CALIBRATION CURVE FOR TELMISARTAN AT 217,225,232 nm

(GEOMETRIC CORRECTTION METHOD)

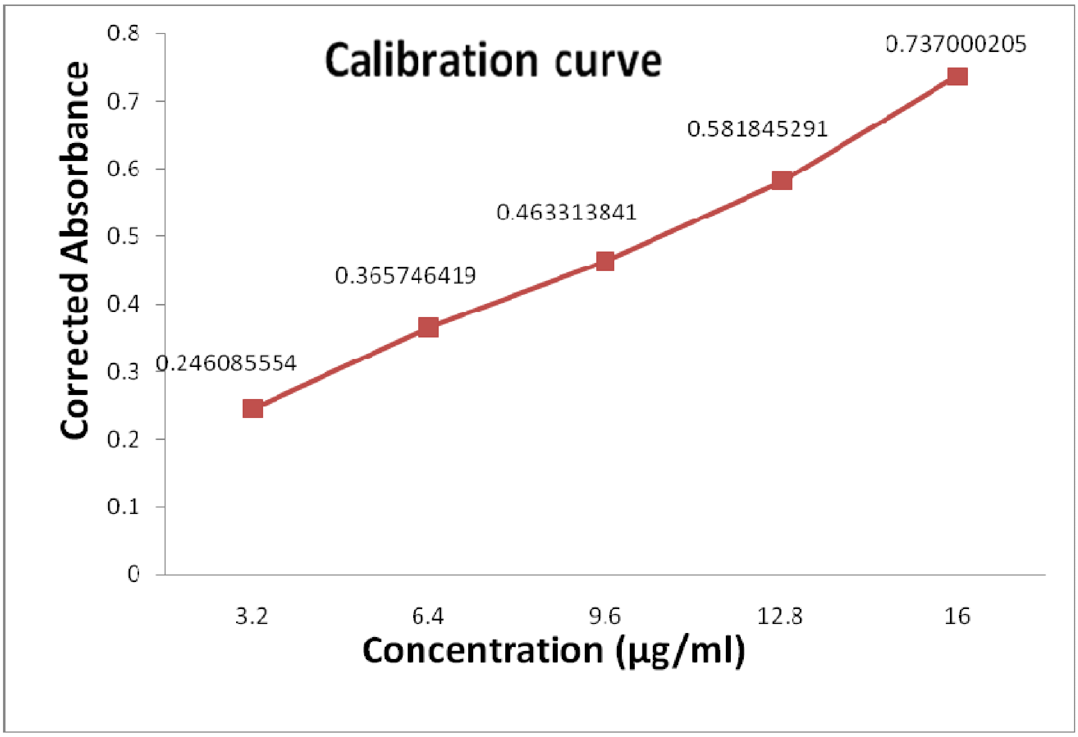


FIGURE-27

CALIBRATION CURVE FOR TELMISARTAN AT 267,275,283 nm

(GEOMETRIC CORRECTTION METHOD)

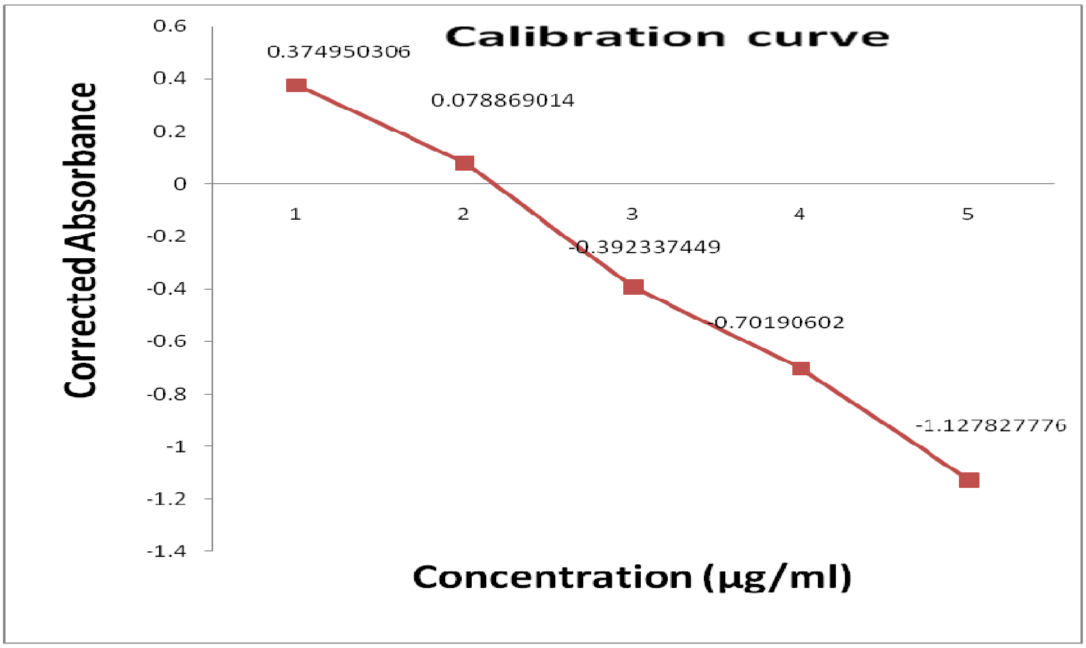


FIGURE-28

WAVELENGTH SELECTION FOR RP-HPLC METHOD

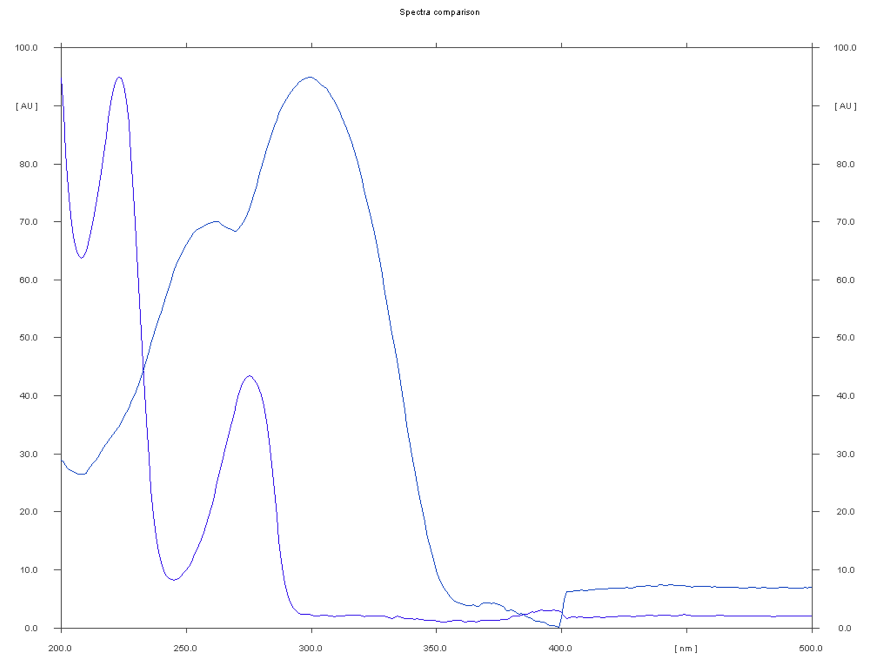
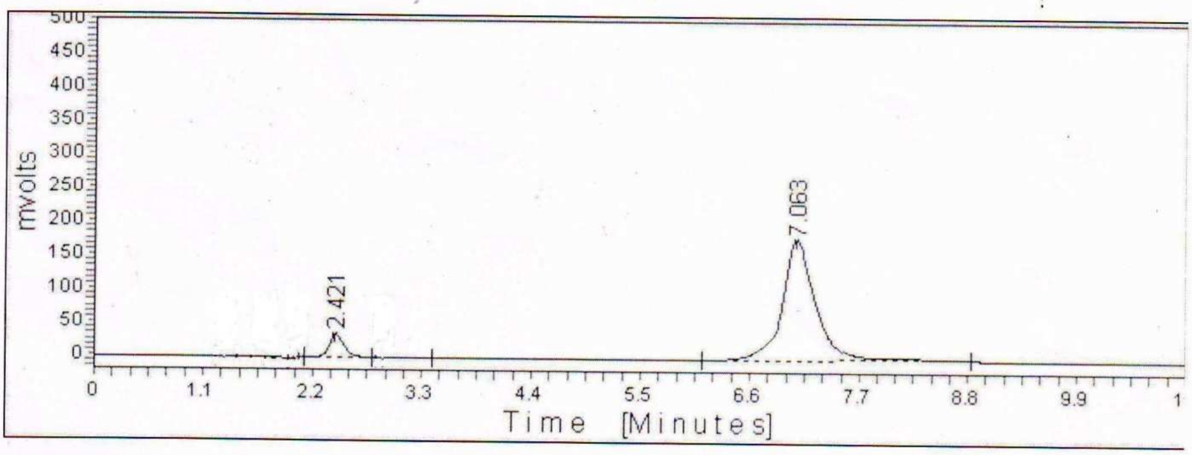


FIGURE-29

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN-1 (30, 48 μgmL^{-1})

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL
Method Name	:	METOPROLOL.MTH
Run Time	:	12 [Minutes.]
Print Time	:	1:38:18 PM



Result-A Table

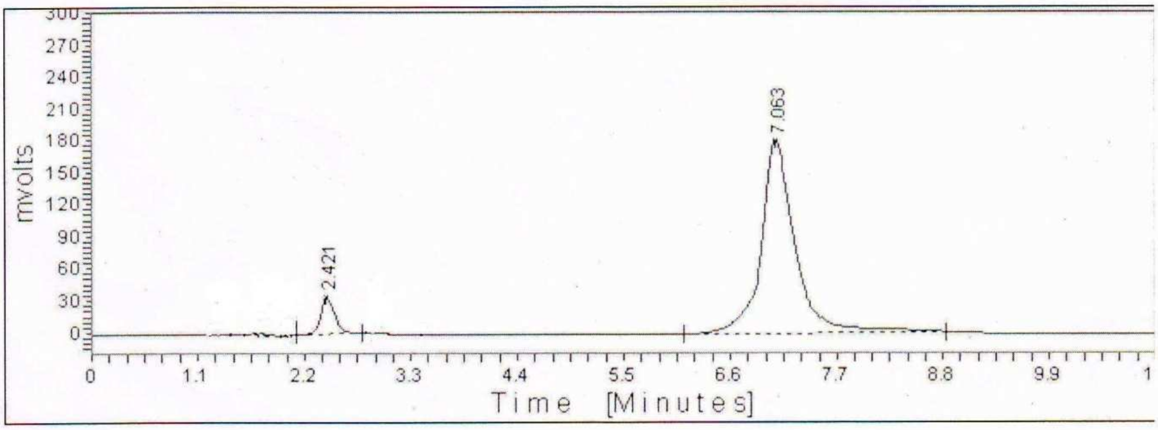
Peak No	Retn.Time	Area	Height	Area %
1	2.421	317.818	36.311	6.885
2	7.063	4007.818	180.89	92.61

FIGURE-30

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN-2 (40, 64 µgmL^{-1})

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL
Method Name	:	METOPROLOL.MTH
Run Time	:	12 [Minutes.]
Print Time	:	4:37:46 PM



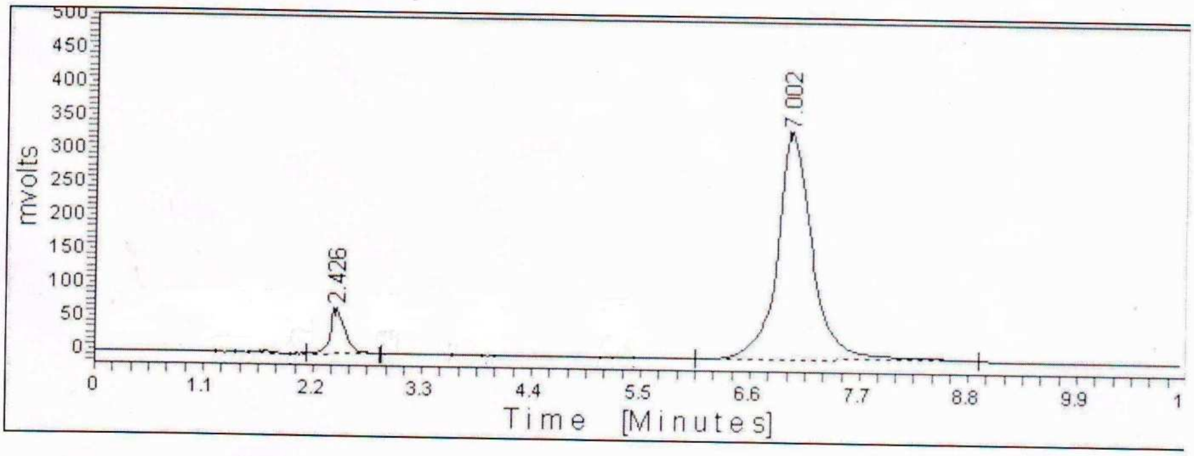
Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.421	468.898	36.311	7.181
2	7.063	5389.746	179.934	92.292

FIGURE-31

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN-3 (50, 80 μgmL^{-1})

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	1:34:25 PM



Result-A Table

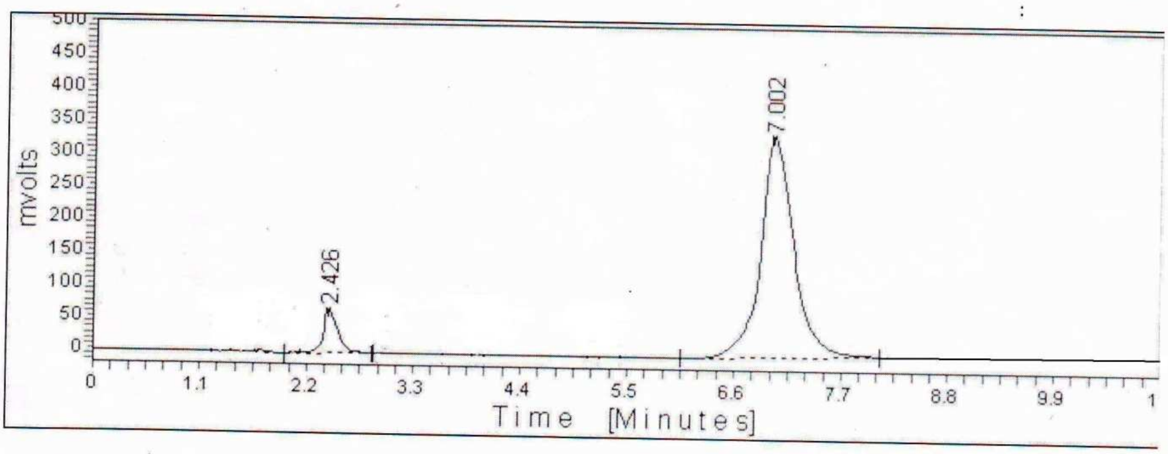
Peak No	Retn.Time	Area	Height	Area %
1	2.426	578.959	70.592	7.31
2	7.002	7012.305	341.187	92.102

FIGURE-32

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN-4 (60, 96 μgmL^{-1})

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL
Method Name	:	METOPROLOL.MTH
Run Time	:	8 [Minutes.]
Print Time	:	1:37:51 PM



Result-A Table

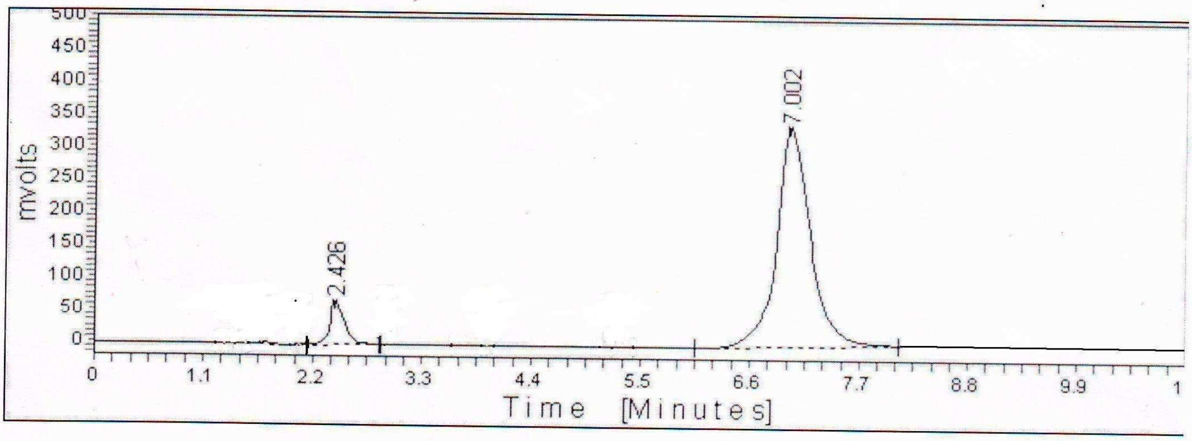
Peak No	Retn.Time	Area	Height	Area %
1	2.426	699.298	70.592	7.686
2	7.002	8193.60	338.83	91.696

FIGURE-33

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN-5 (70, 112 μgmL^{-1})

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL-
Method Name	:	METOPROLOL.MTH
Run Time	:	8 [Minutes.]
Print Time	:	1:38:03 PM



Result-A Table

Peak No	Retn.Time	Area	Height	Area %
1	2.426	820.642	70.592	7.686
2	7.002	9808.637	338.83	91.696

FIGURE-34

CALIBRATION CURVE OF METOPROLOL SUCCINATE BY RP – HPLC METHOD

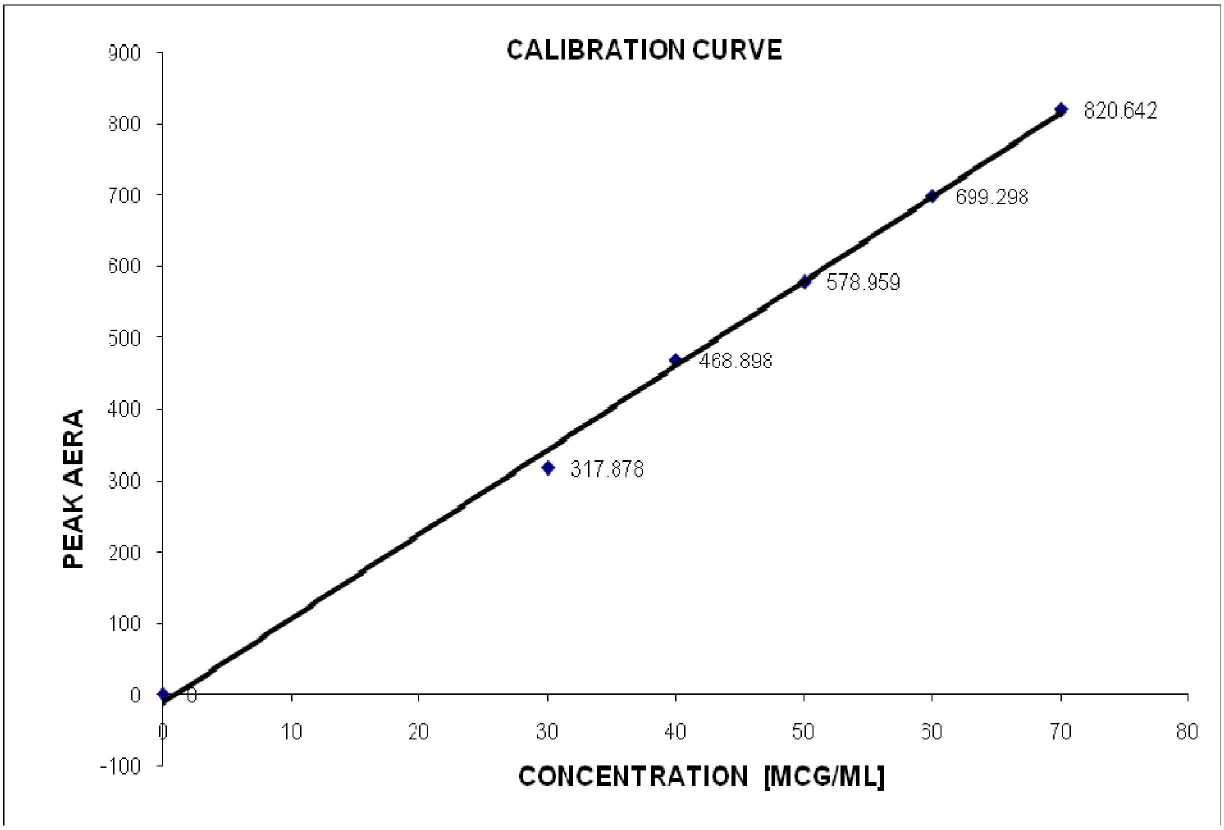


FIGURE-35

CALIBRATION CURVE OF TELMISARTAN BY RP – HPLC METHOD

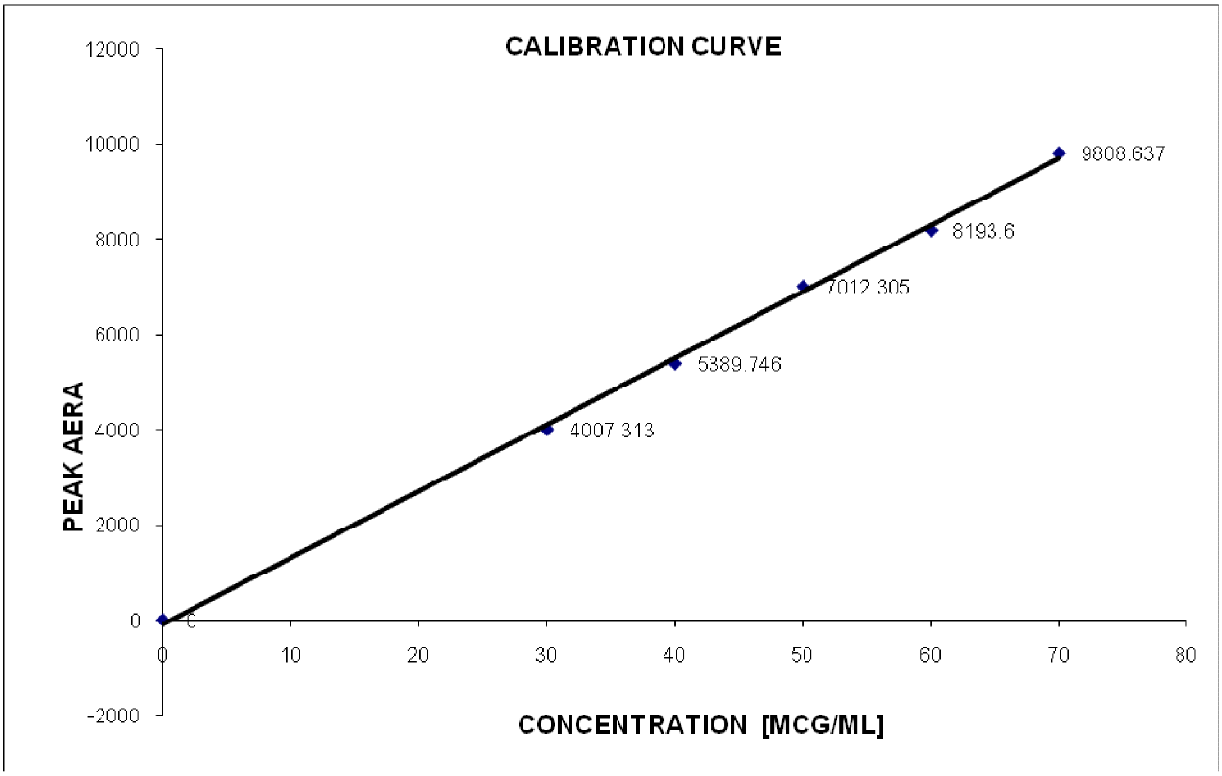
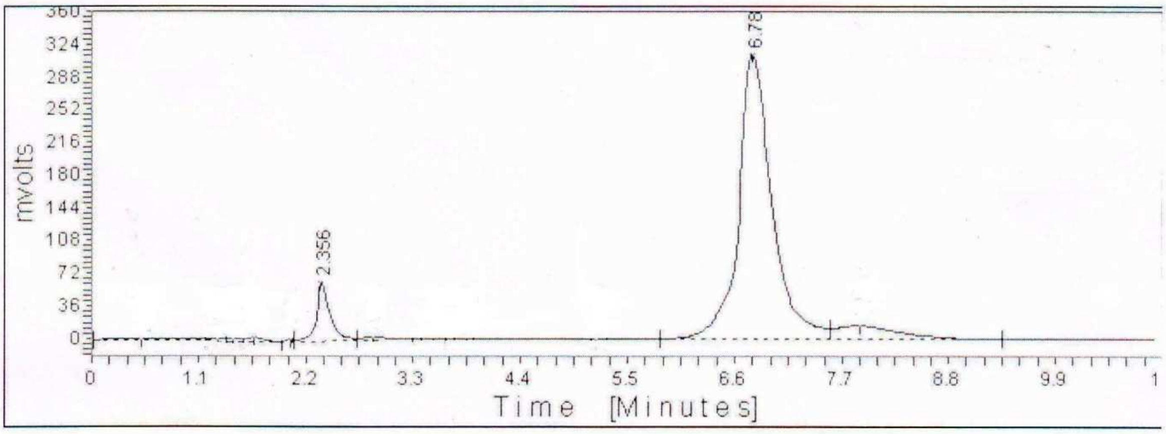


FIGURE-36

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 1

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 01_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	9:39:28 AM



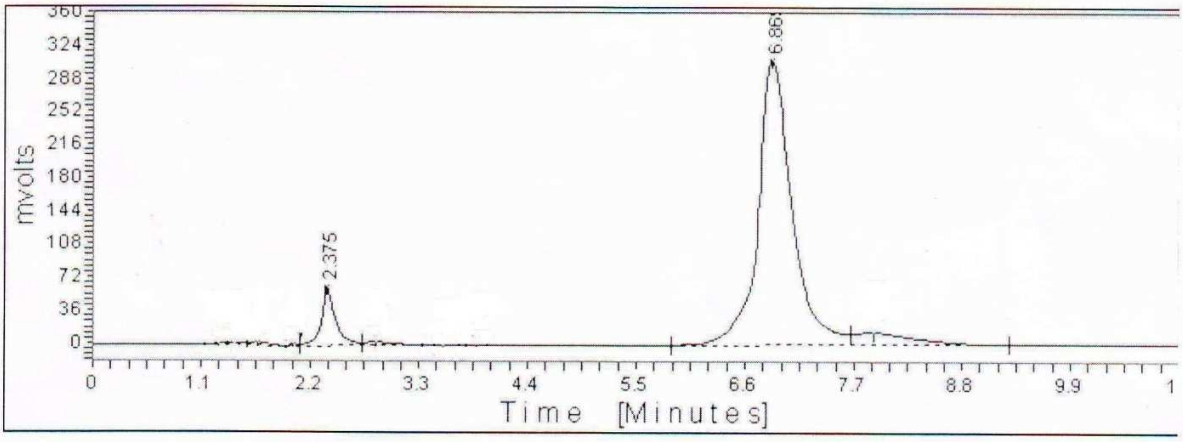
Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.356	591.53	66.202	6.806
2	6.782	7148.946	313.81	82.252

FIGURE-37

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 2

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 02_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	13 [Minutes.]
Print Time	:	9:53:57 AM



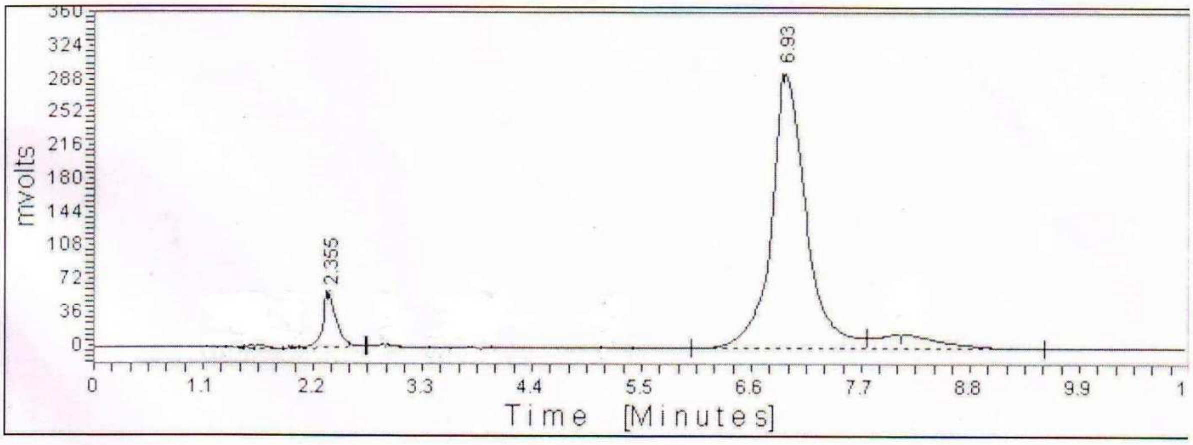
Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.375	597.344	66.908	7.005
2	6.869	7181.08	311.231	84.208

FIGURE-38

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 3

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	4:50:33 PM



Result-A Table

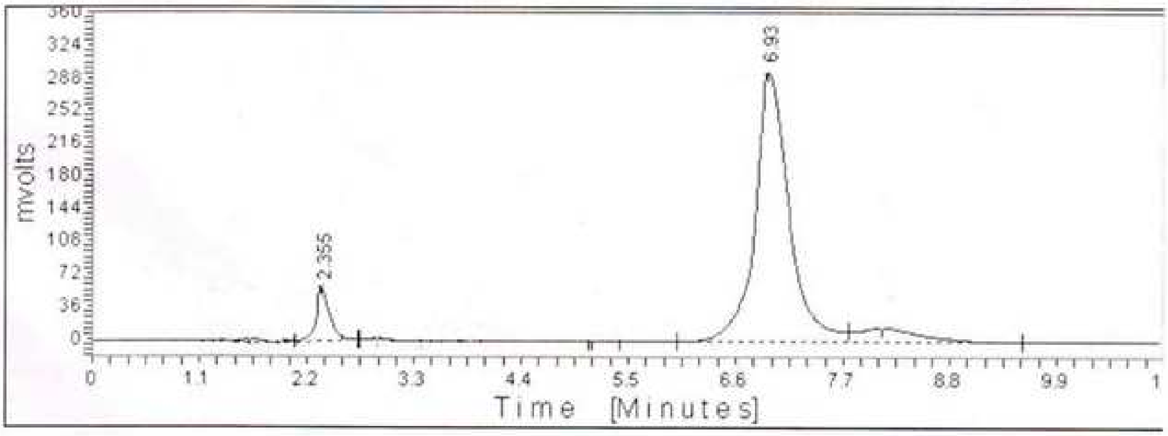
Peak No	Retn.Time	Area	Height	Area %
1	2.355	596.056	61.673	6.295
2	6.93	7156.985	298.596	83.933

FIGURE-39

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 4

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	4:51:33 PM



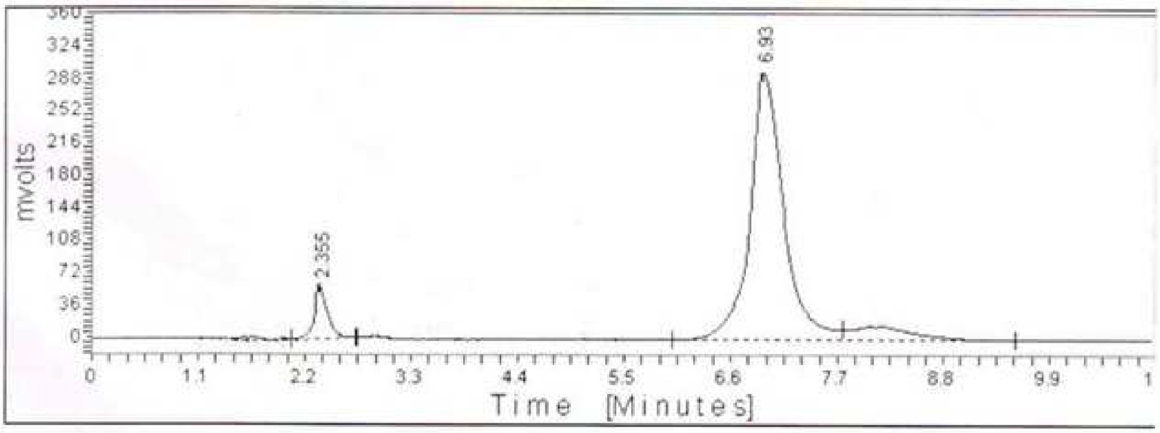
Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.355	593.859	61.673	6.295
2	6.93	7196.896	298.596	83.933

FIGURE-40

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 5

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	4:52:33 PM



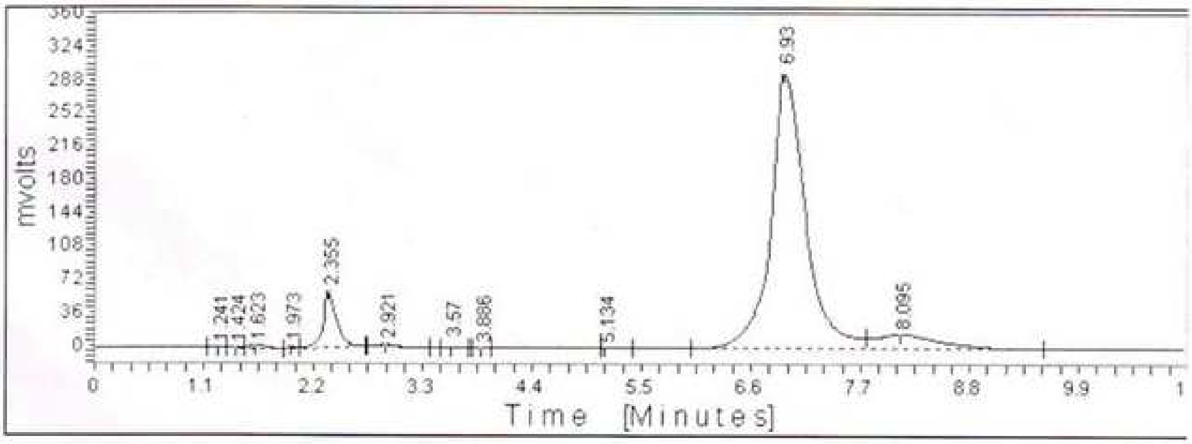
Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.355	590.463	61.673	6.295
2	6.93	7169.589	298.596	83.933

FIGURE-41

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 6

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO ASSAY 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	4:55:33 PM



Result-A Table				
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**
1	2.355	591.257	61.673	6.295
2	6.93	7209.561	298.596	83.933

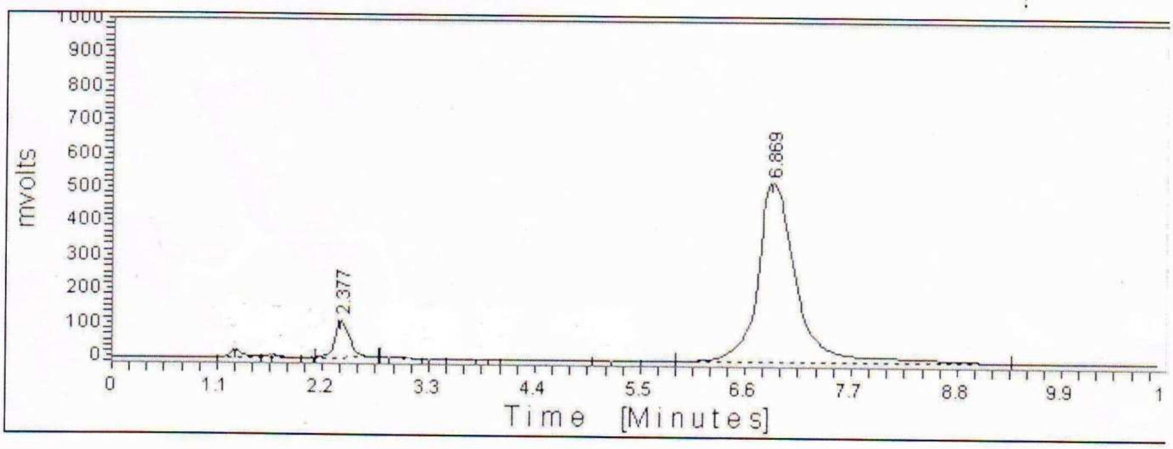
FIGURE – 42

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)

RECOVERY-1

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO 80 REC 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	10:35:08 AM



Result-A Table

Peak No	Retn.Time	Area	Height	Area %
1	2.377	1068.153	110.701	6.746
2	6.869	12979.694	529.419	91.077

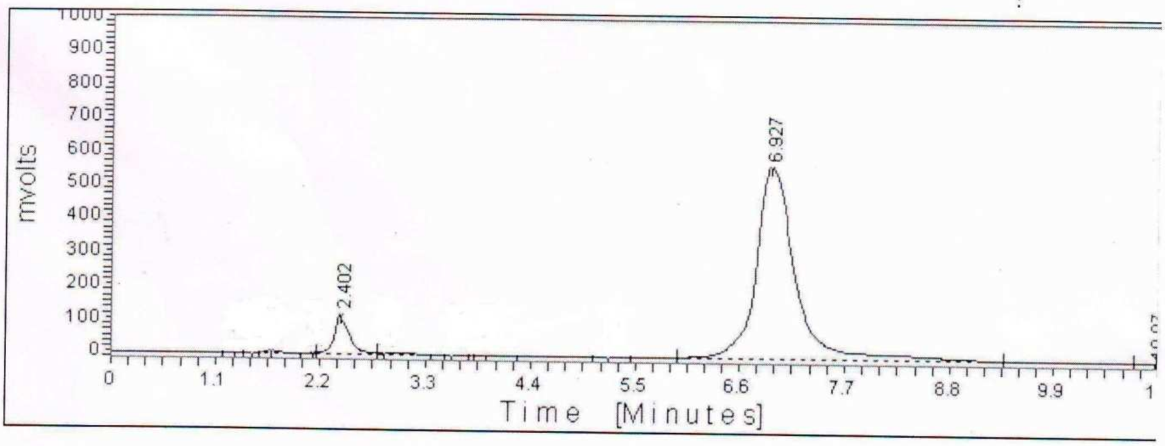
FIGURE – 43

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)

RECOVERY-2

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO 100 REC 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	11:01:22 AM



Result-A Table

Peak No	Retn.Time	Area	Height	Area %
1	2.402	1186.836	118.672	7.219
2	6.927	14421.882	568.225	91.457

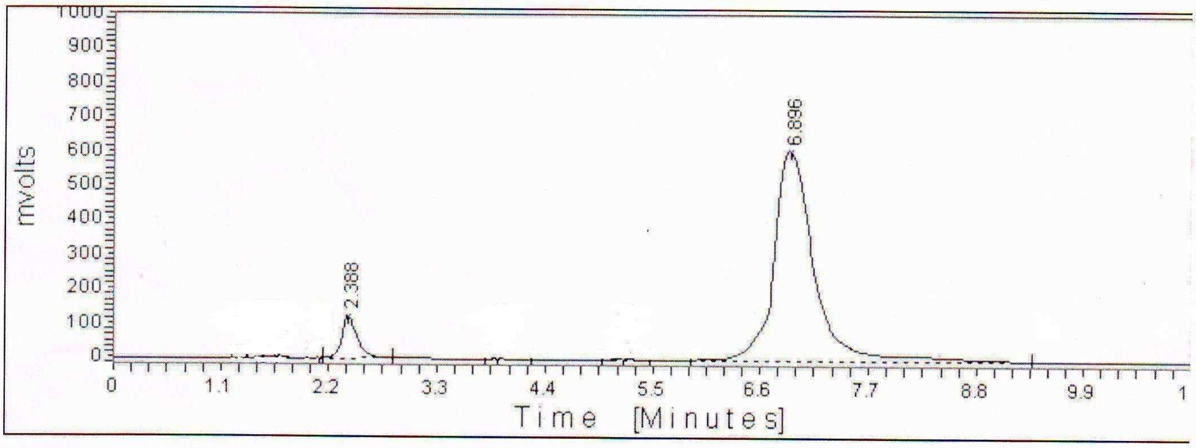
FIGURE – 44

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)

RECOVERY-3

BONN SCHTERING BIO SCIENCES-PUDUCHERRY-605 107

File Name	:	DataAce - CH-I Chromatogram...DataFileName : e:\hplc analysis\METOPROLOL\\TEL+METO 120 REC 1_1.cmg
Method Name	:	METOPROLOL.MTH
Run Time	:	11 [Minutes.]
Print Time	:	10:48:13 AM



Result-A Table

Peak No	Retn.Time	Area	Height	Area %
1	2.388	1306.391	124.509	6.899
2	6.896	15864.070	610.561	92.568

FIGURE-45

WAVELENGTH SELECTION FOR HPTLC METHOD

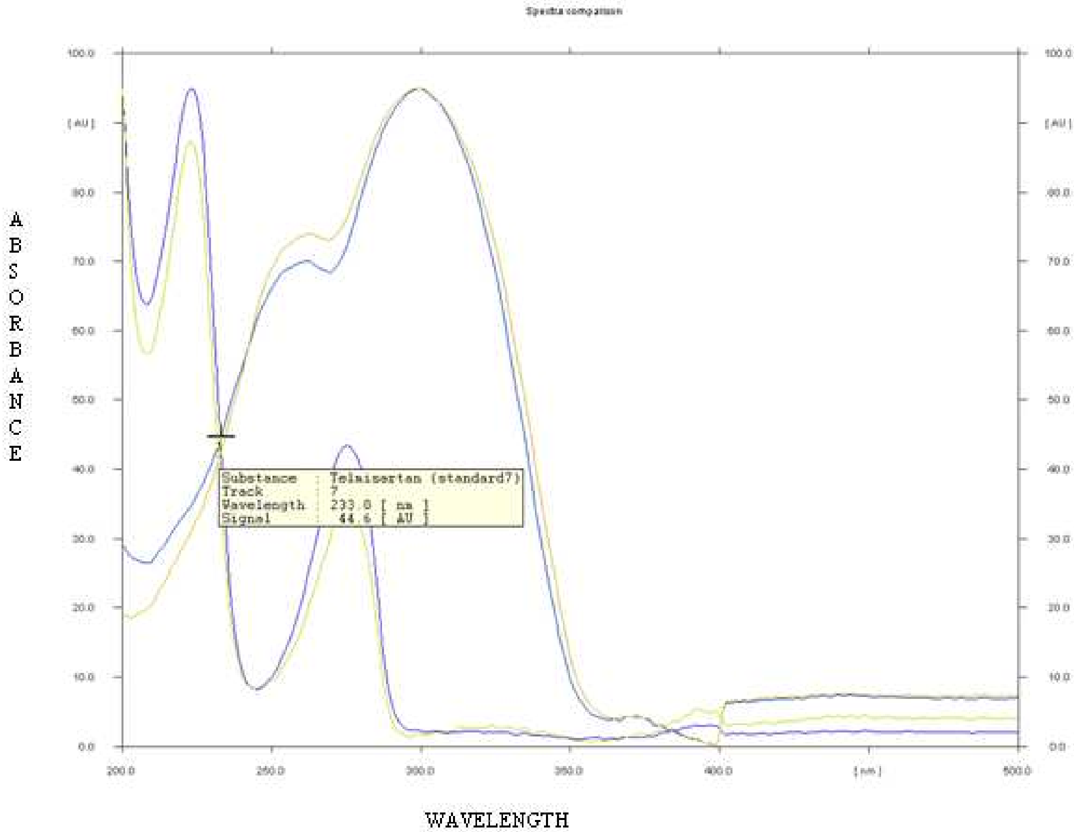


FIGURE –46

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD-1 [1, 1.6 μgmL^{-1}]

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.45	4.1	0.48	39.6	45.33	0.50	8.1	846.0	39.14	Metoprolol
2	0.58	19.9	0.61	47.8	54.67	0.66	3.6	1315.5	60.86	Telmisartan

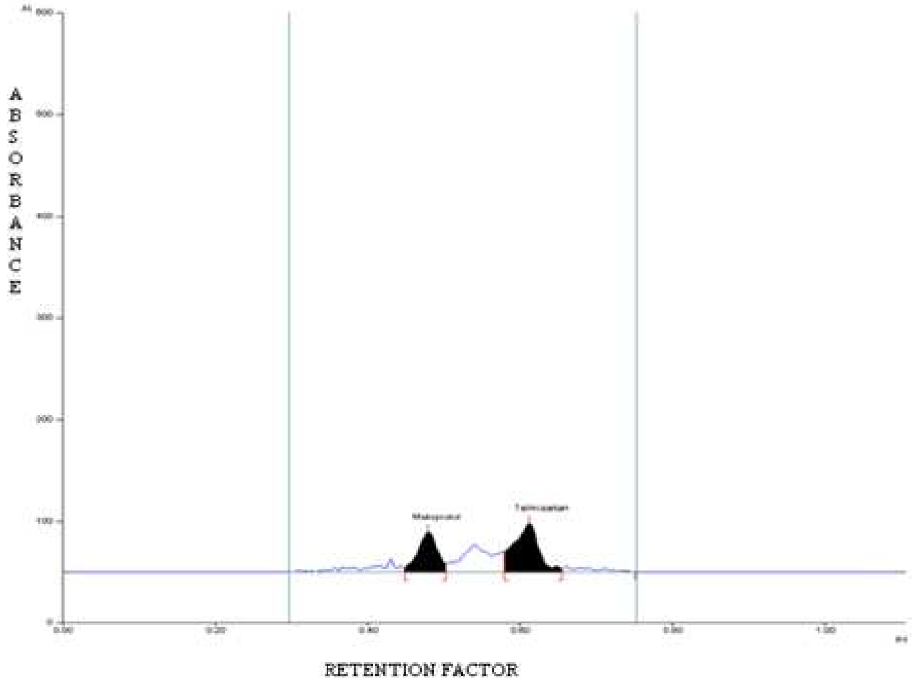


FIGURE –47

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD [2, 3.2 μgmL^{-1}]

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	6.2	0.45	74.7	49.66	0.48	10.9	1356.1	45.41	Metoprolol
2	0.54	19.3	0.58	75.7	50.34	0.62	3.3	1630.1	54.59	Telmisartan

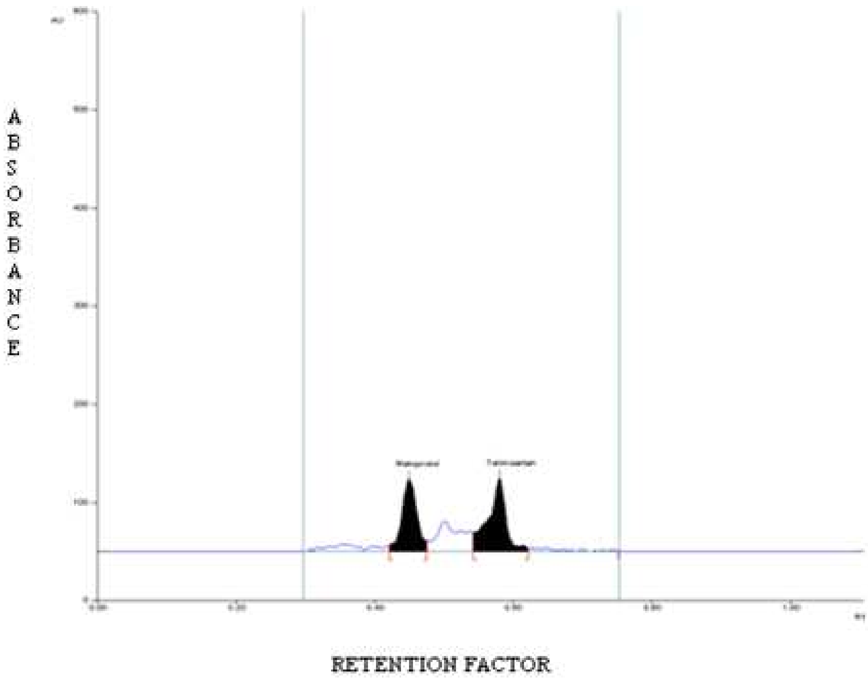


FIGURE –48

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD [3, 4.8 µgmL^{-1}]

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	8.5	0.45	111.4	51.25	0.47	15.7	2140.5	49.02	Metoprolol
2	0.54	23.8	0.58	106.0	48.75	0.61	11.5	2225.9	50.98	Telmisartan

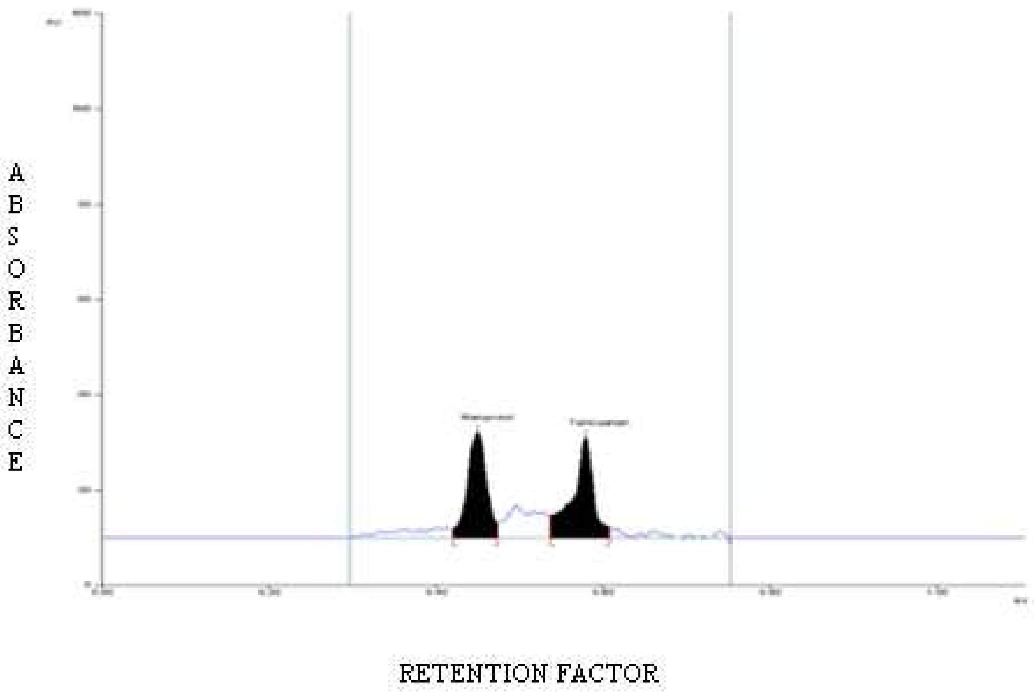


FIGURE –49

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD [4, 6.4 μgmL^{-1}]

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	6.2	0.45	132.7	51.80	0.47	17.3	2440.0	47.41	Metoprolol
2	0.54	24.6	0.58	123.5	48.20	0.60	12.0	2799.4	52.59	Telmisartan

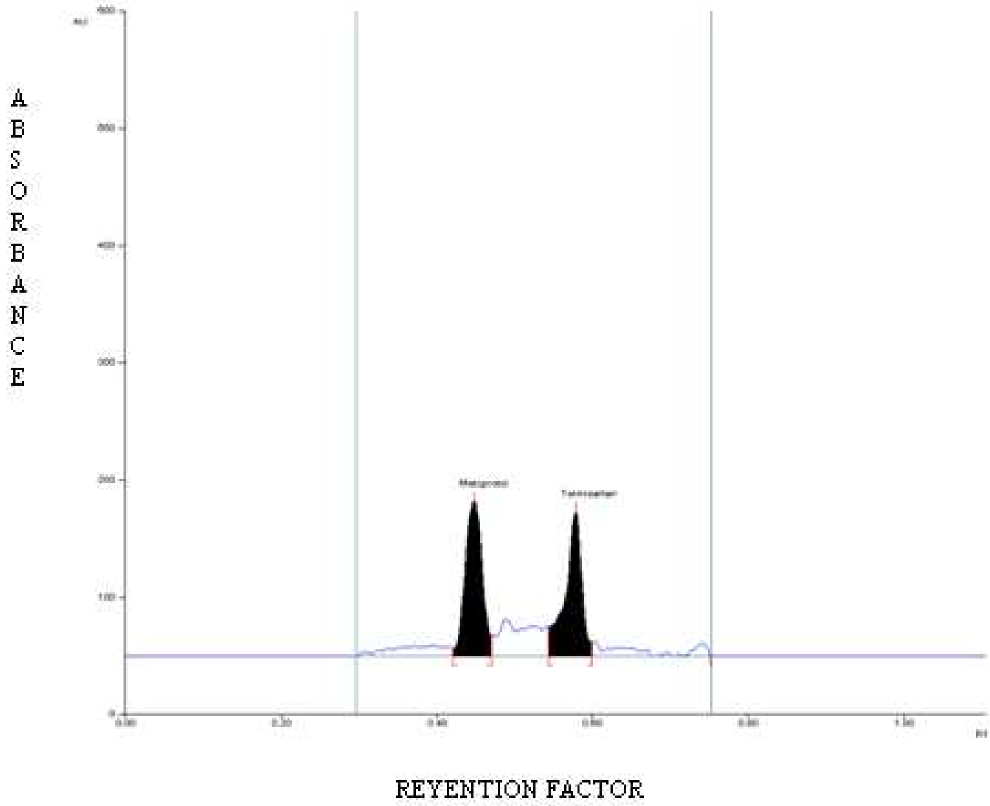


FIGURE –50

LINEARITY CHROMATOGRAM OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD [5, 8 μgmL^{-1}]

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.43	13.9	0.46	184.9	53.10	0.49	15.9	3032.6	43.12	Metoprolol
2	0.55	26.7	0.58	163.3	46.90	0.63	3.8	4000.9	56.88	Telmisartan

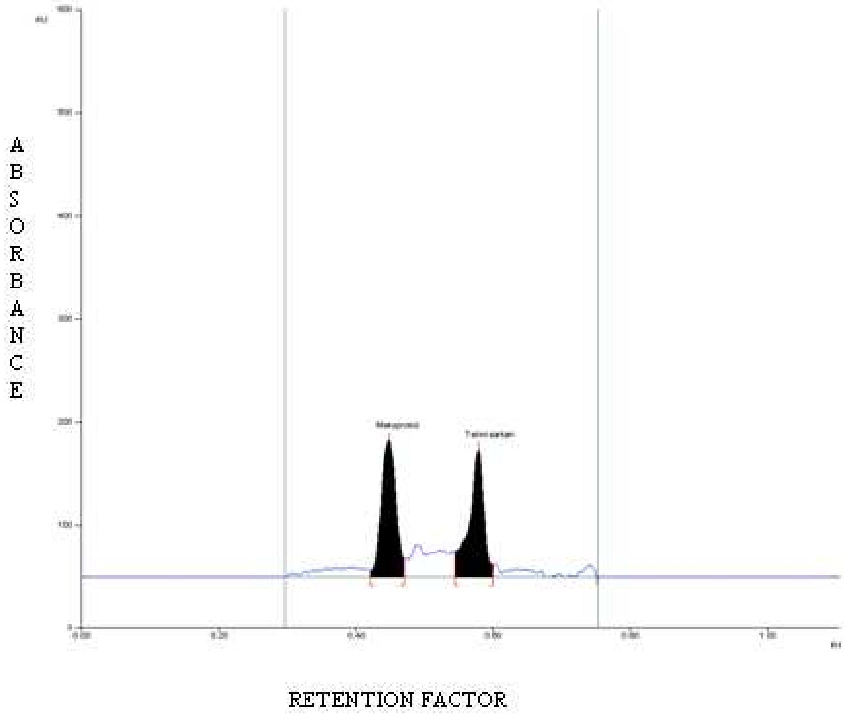


FIGURE –51

SPECTRUM OF METOPROLOL SUCCINATE AND TELMISARTAN IN ALL THE TRACKS

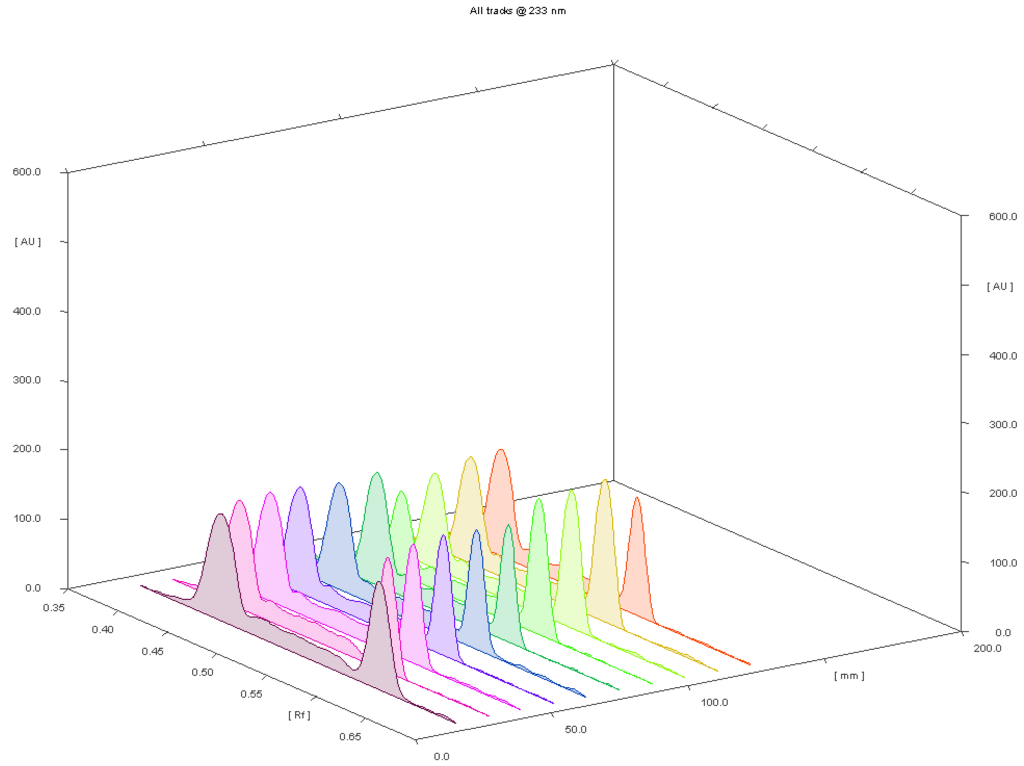


FIGURE –52

CALIBRATION CURVE OF METOPROLOL SUCCINATE AT 233 NM BY HPTLC METHOD

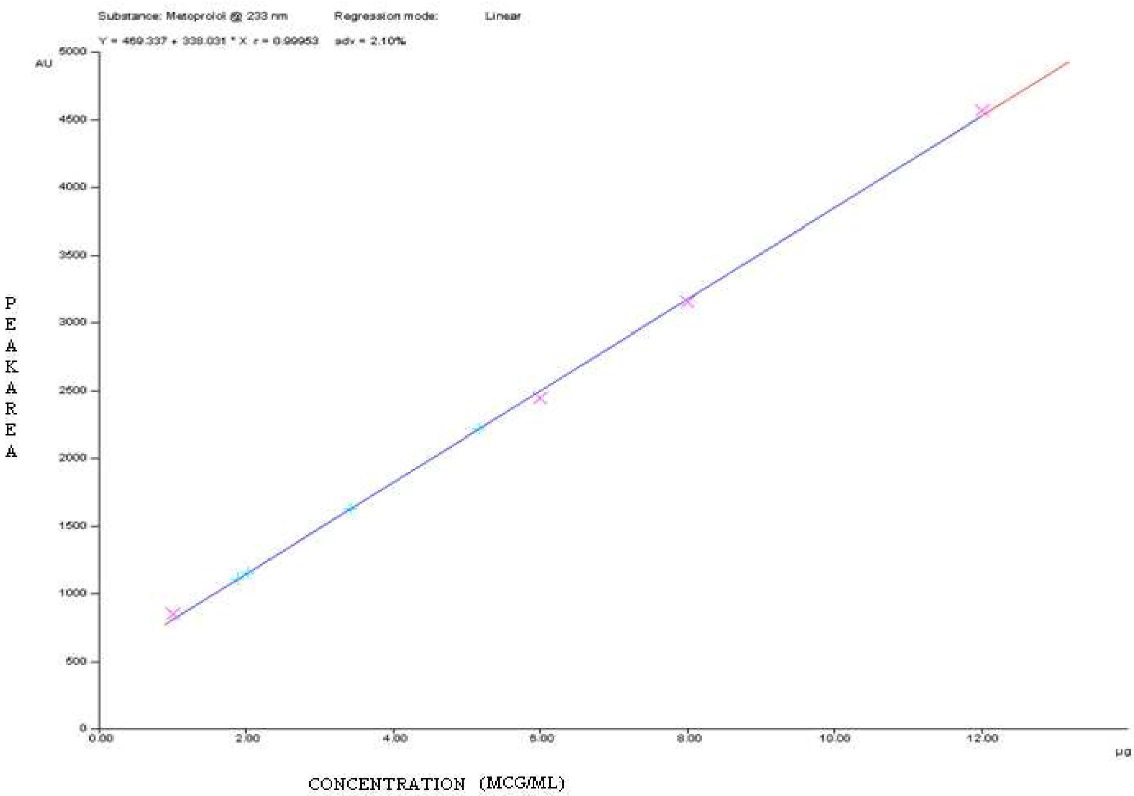


FIGURE –53

CALIBRATION CURVE OF TELMISARTAN AT 233 NM BY HPTLC METHOD

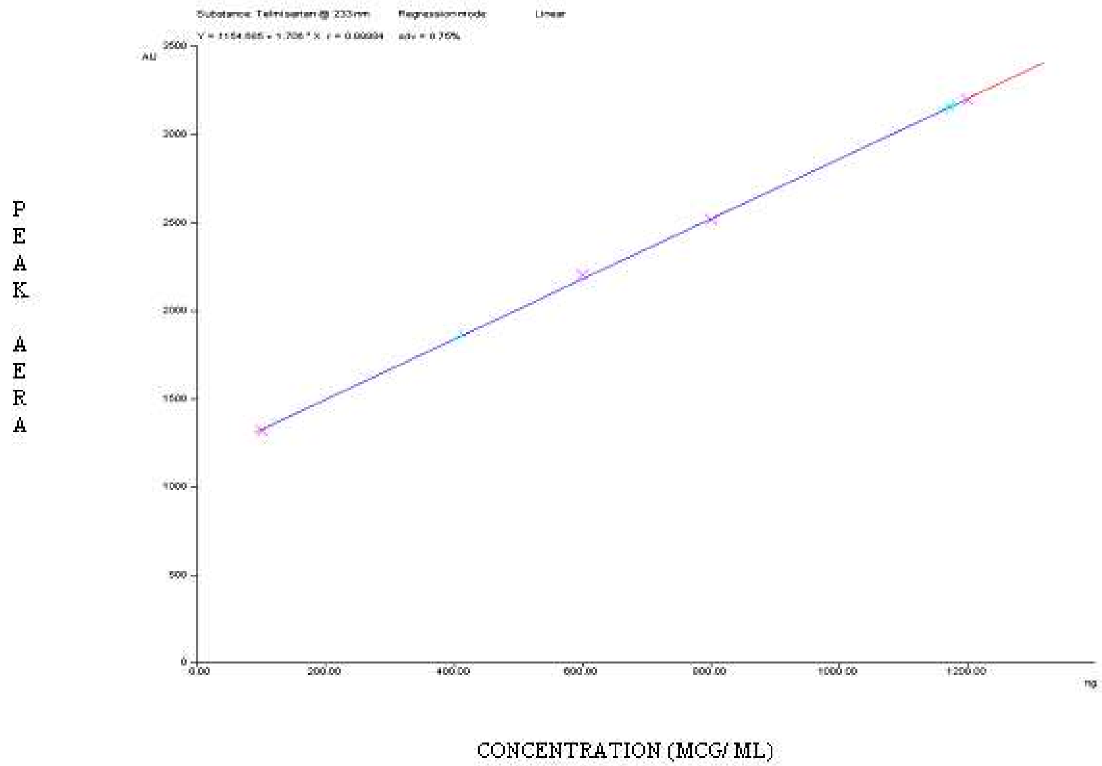


FIGURE-54

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25) REPEATABILITY- 1

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	14.8	0.44	47.2	26.53	0.47	19.9	1336.9	25.97	Metoprolol
2	0.54	30.5	0.58	130.7	73.47	0.63	12.9	1645.1	74.03	Telmisartan

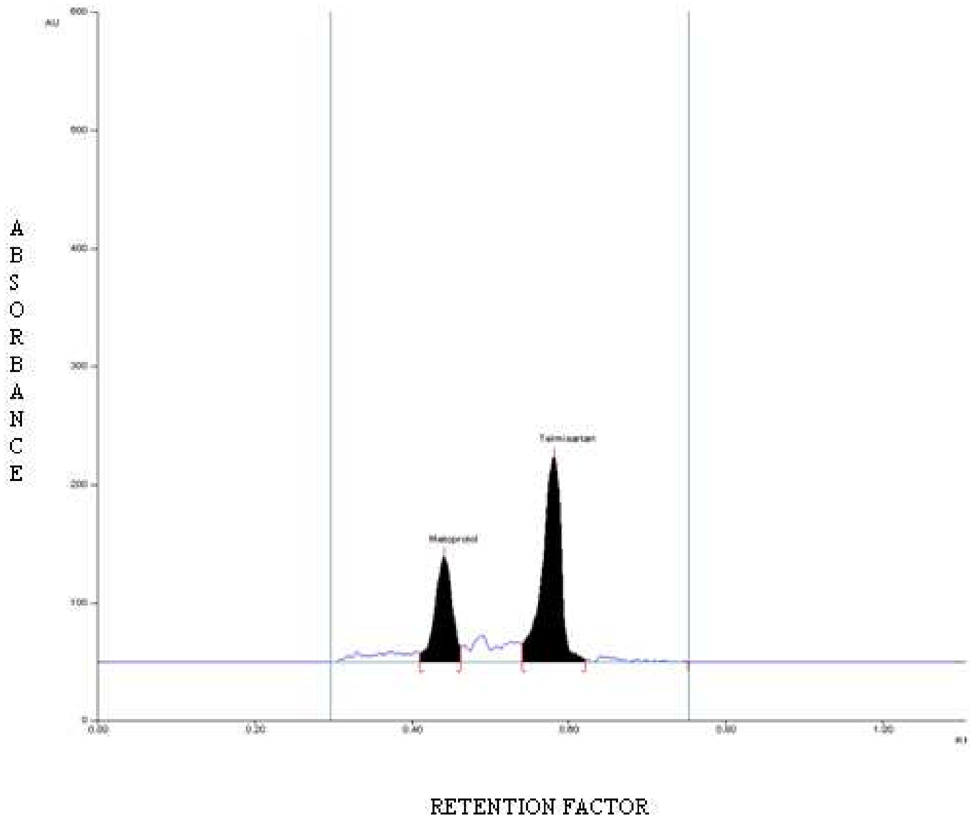


FIGURE-55

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25) REPEATABILITY- 2

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	14.8	0.44	47.2	26.53	0.47	19.9	1340.1	25.97	Metoprolol
2	0.54	30.5	0.58	130.7	73.47	0.63	12.9	1649.3	74.03	Telmisartan

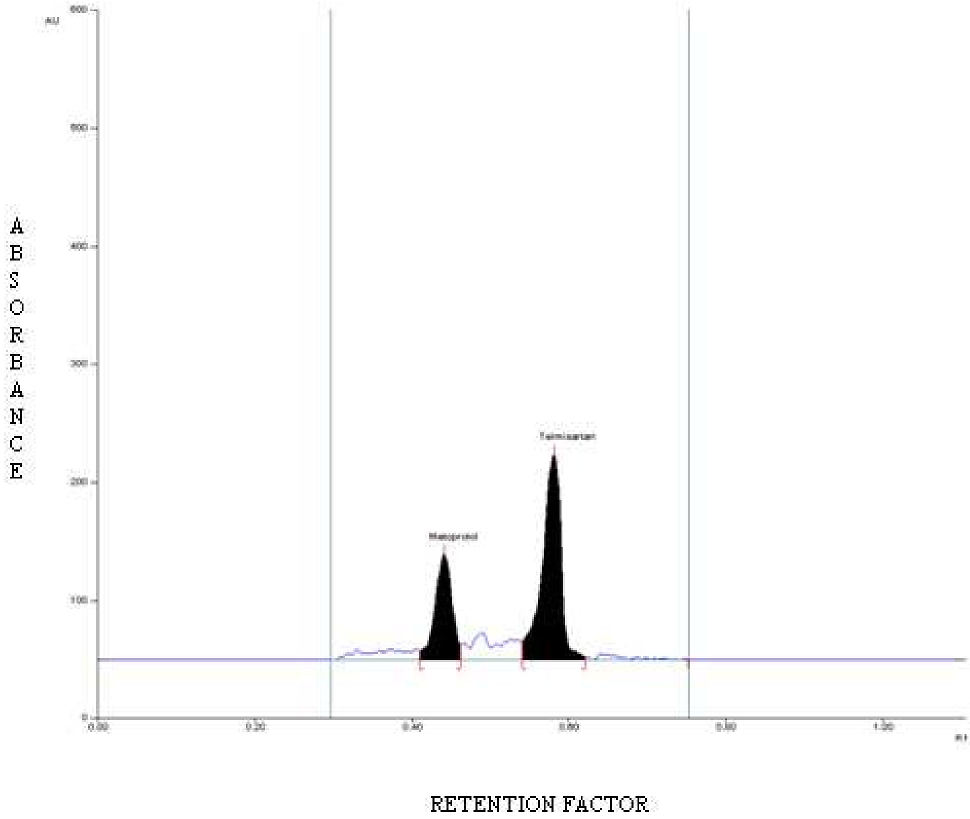


FIGURE-56

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 3

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.40	6.5	0.44	57.9	27.87	0.46	10.3	1336.9	26.76	Metoprolol
2	0.53	16.9	0.58	149.8	72.13	0.62	6.7	1640.7	73.24	Telmisartan

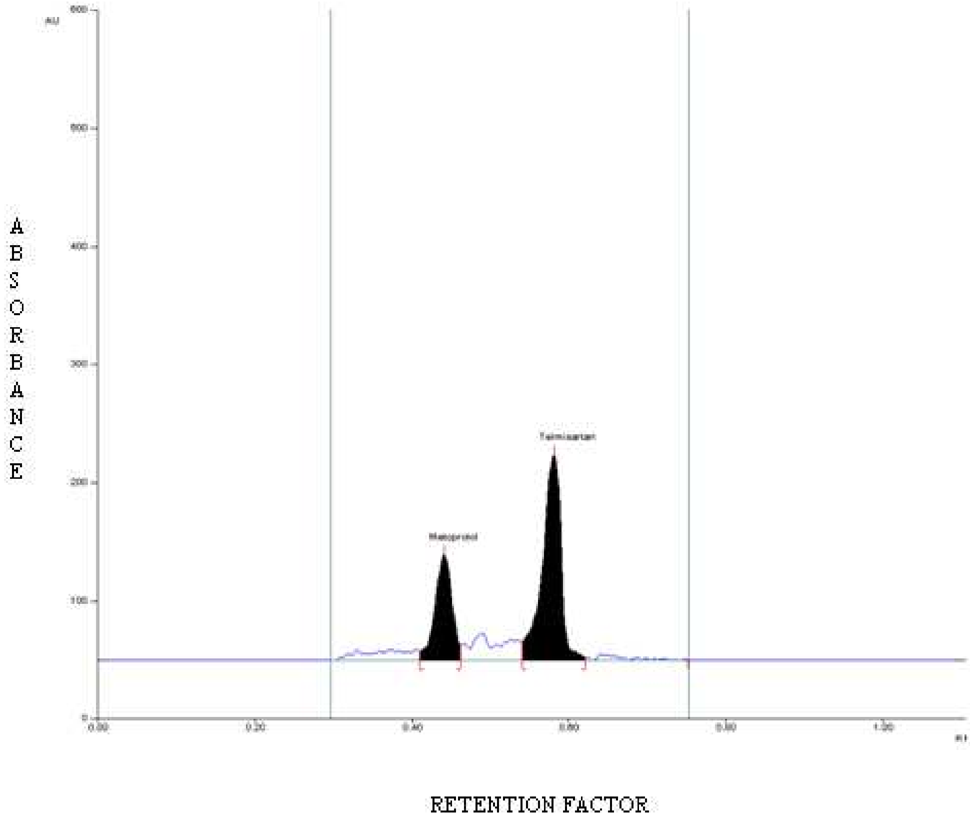


FIGURE-57

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- **4**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	14.8	0.44	47.2	26.53	0.47	19.9	1349.3	25.97	Metoprolol
2	0.54	30.5	0.58	130.7	73.47	0.63	12.9	1635.3	74.03	Telmisartan

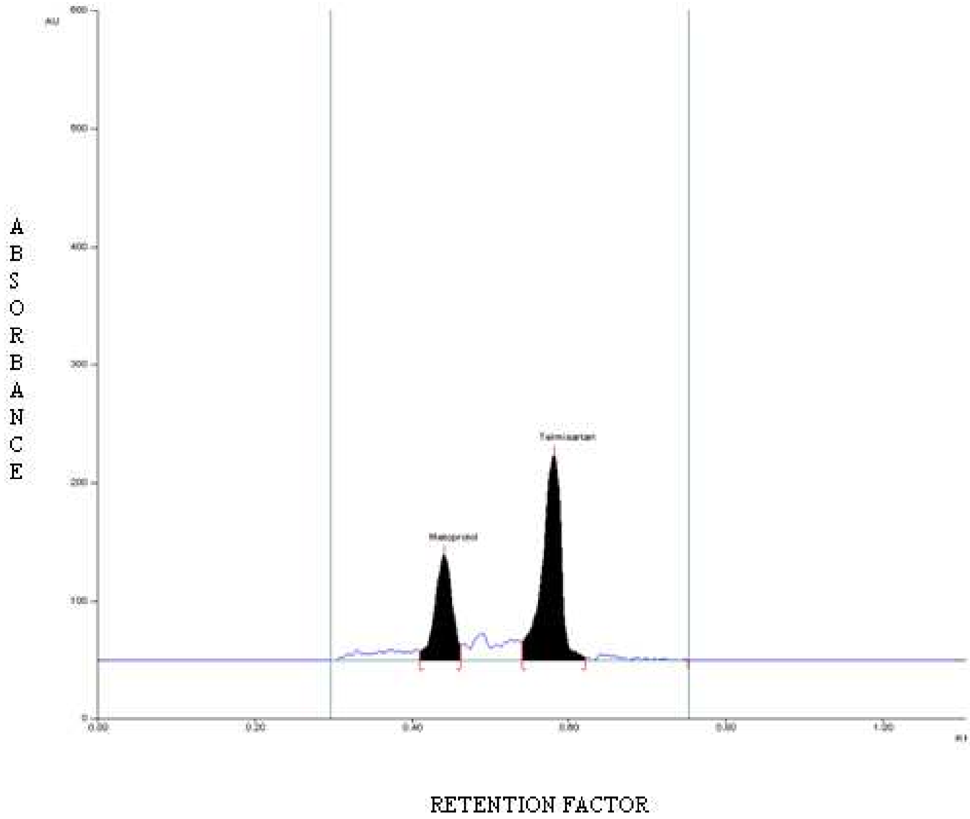


FIGURE-58

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 5

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.40	6.5	0.44	57.9	27.87	0.46	10.3	1351.0	26.76	Metoprolol
2	0.53	16.9	0.58	149.8	72.13	0.62	6.7	1673.5	73.24	Telmisartan

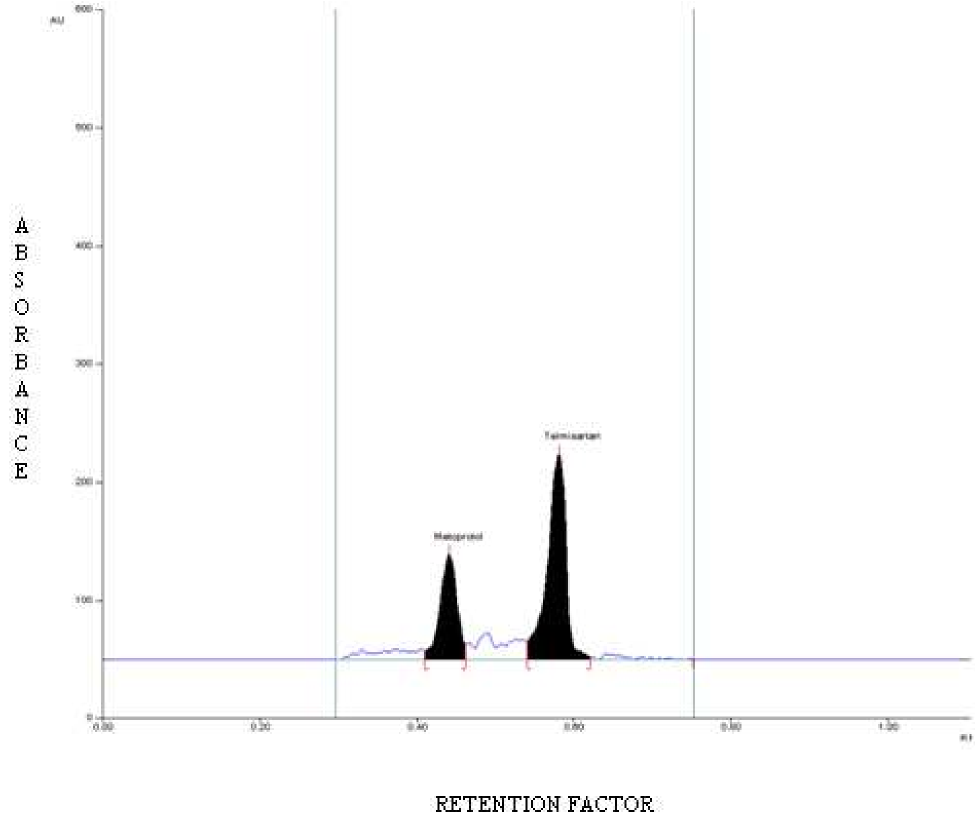


FIGURE-59

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)
REPEATABILITY- 6

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	14.8	0.44	47.2	26.53	0.47	19.9	1331.7	25.97	Metoprolol
2	0.54	30.5	0.58	130.7	73.47	0.63	12.9	1630.8	74.03	Telmisartan

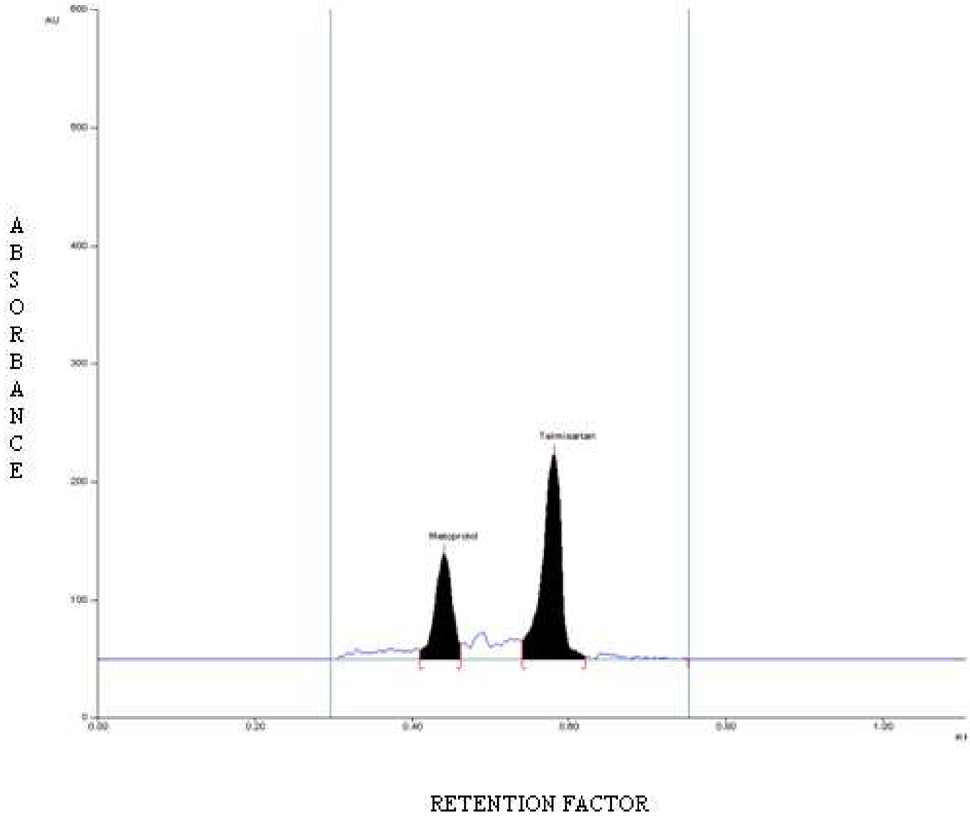


FIGURE – 60

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25) 80%

RECOVERY-1

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	6.2	0.45	74.7	49.66	0.48	10.9	1775.9	45.41	Metoprolol
2	0.54	19.3	0.58	75.7	50.34	0.62	3.3	1830.2	54.59	Telmisartan

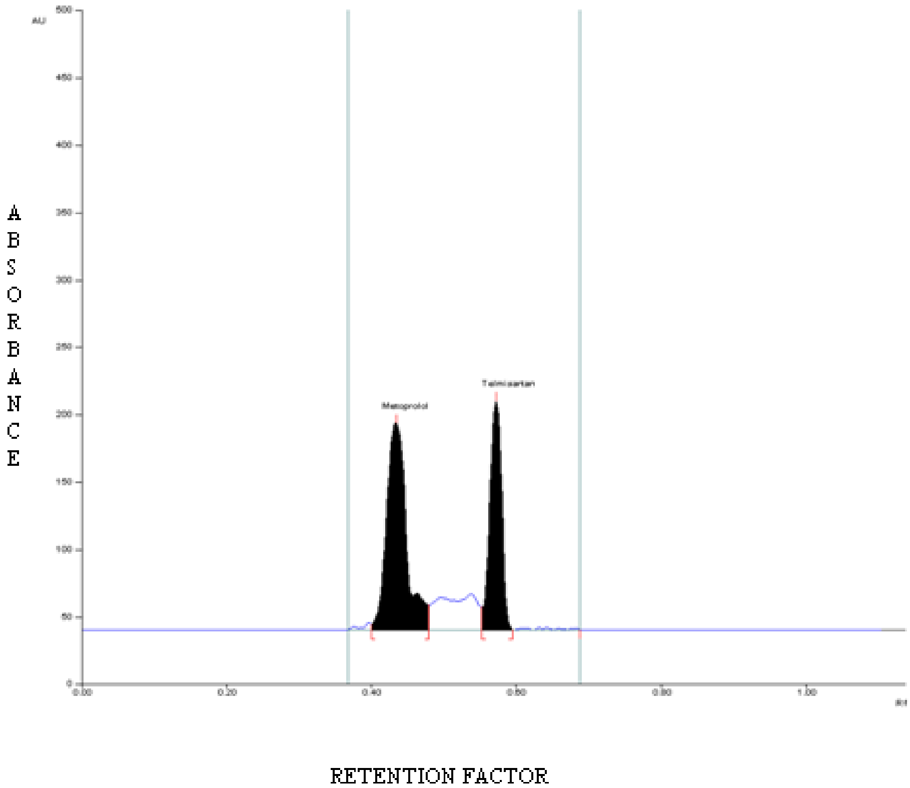


FIGURE – 61

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25) 100%

RECOVERY-2

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	7.4	0.44	88.7	33.74	0.46	12.9	2004.1	31.82	Metoprolol
2	0.54	15.4	0.58	174.2	66.26	0.62	2.4	2159.2	68.18	Telmisartan

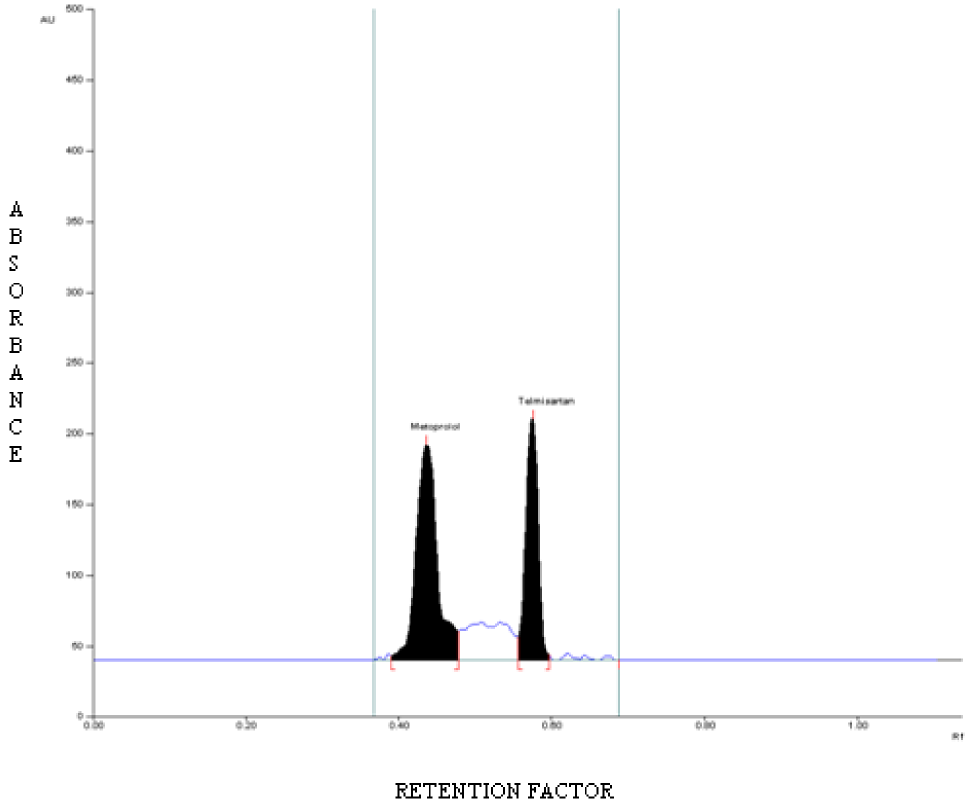


FIGURE – 62

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (METOSARTAN-25)

RECOVERY-3

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	5.1	0.45	106.6	36.46	0.49	10.1	2271.3	37.41	Metoprolol
2	0.55	16.2	0.59	185.8	63.54	0.63	1.4	2488.2	62.59	Telmisartan

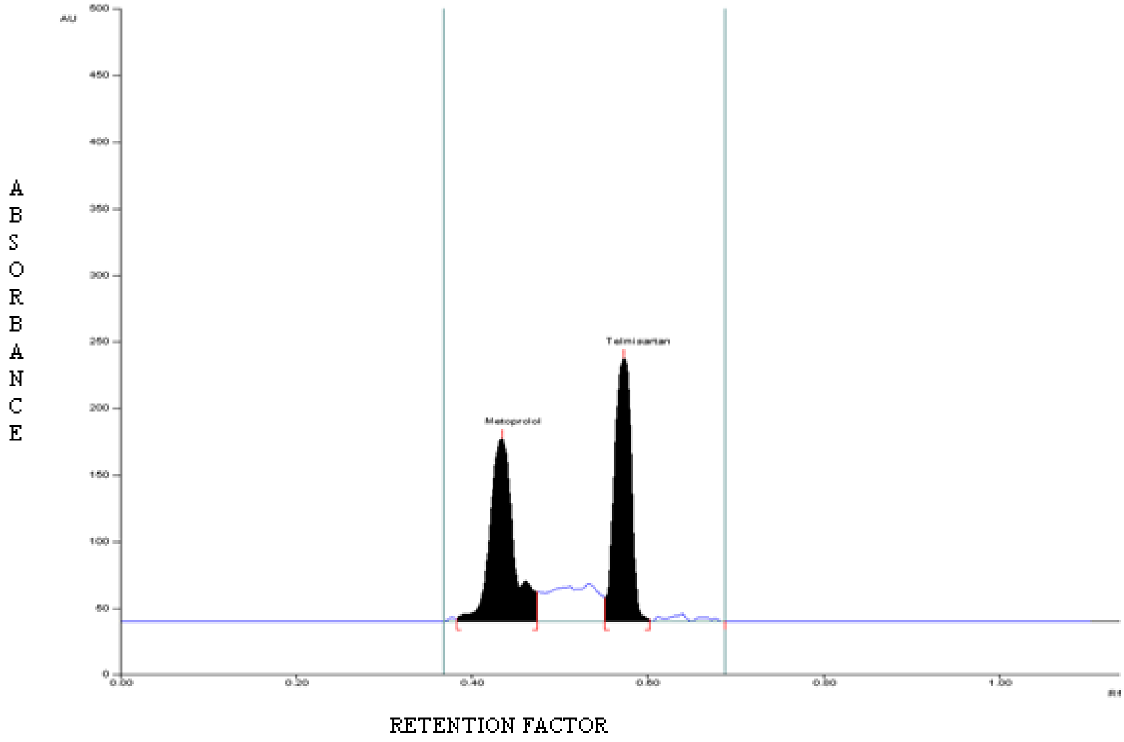
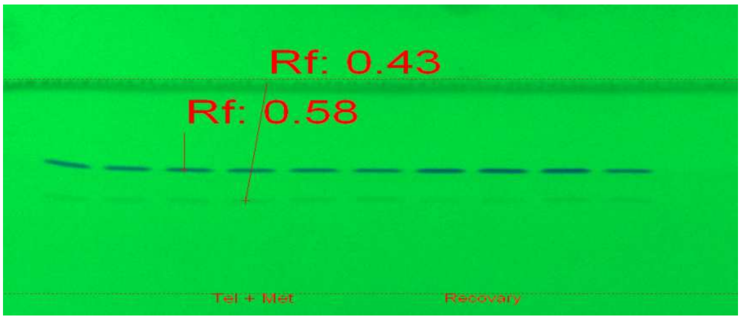


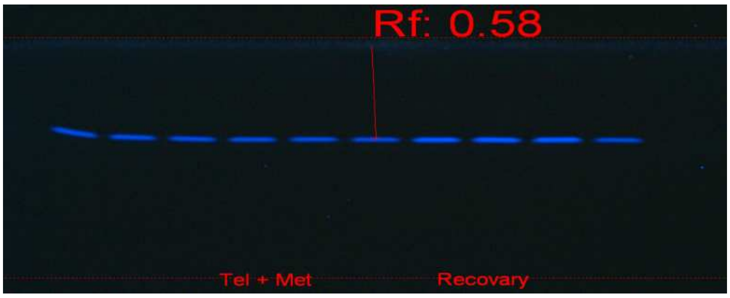
FIGURE-63

DIFFERENCE BETWEEN SHORTER AND LONGER WAVELENGTH OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD

Shorter wavelength at 254 nm



Longer wavelength at 366 nm



8. TABLES

TABLE-1

SOLUBILITY PROFILE OF METOPROLOL SUCCINATE

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	SOLUBILITY STATUS
1	Distilled water	10 mg in .02 ml	Freely soluble
2	0.1M Hydrochloric acid	10 mg in 0.2 ml	Soluble
3	0.1M Sodium Hydroxide	10 mg in 0.5 ml	Sparingly soluble
4	Methanol	10 mg in 0.3ml	Soluble
5	Ethanol	10 mg in 1.4 ml	Slightly soluble
6	n – Butanol	10 mg in 0.6 ml	Sparingly soluble
7	Chloroform	10 mg in 0.7 ml	Sparingly soluble
8	DMF	10 mg in 5 ml	Slightly soluble
9	Acetone	10 mg in 2 ml	Slightly soluble
10	Dilute acetic acid	10 mg in 0.4 ml	Sparingly soluble
11	Ethyl acetate	10 mg in 0.5 ml	Sparingly soluble
12	Petroleum ether	10 mg in MT 10 ml	Insoluble
13	Toluene	10 mg in 10 ml	Insoluble
14	Carbon tetra chloride	10 mg in 5 ml	Slightly soluble
15	Acetonitrile	10 mg in 3 ml	Slightly soluble
16	Benzene	10 mg in MT 10 ml	Insoluble
17	Dichloromethane	10 mg in 9 ml	Slightly soluble
18	n-Hexane	10 mg in MT 10 ml	Insoluble
19	IPA	10 mg in 3 ml	Slightly soluble
20	Acetate buffer pH-3.5	10 mg in MT100 ml	Insoluble
21	Acetate buffer pH-6.0	10 mg in MT 100 ml	Insoluble
22	Phosphate buffer pH – 6.0	10 mg in 1 ml	Sparingly soluble
23	Phosphate buffer pH – 7.0	10 mg in 0.5ml	Sparingly soluble
24	Phosphate buffer pH – 8.0	10 mg in 0.2 ml	Soluble

TABLE-2

SOLUBILITY PROFILE OF TELMISARTAN

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	SOLUBILITY STATUS
1	Distilled water	10 mg in MT100 ml	In soluble
2	0.1M Hydrochloric acid	10 mg in 2.5 ml	Slightly soluble
3	0.1M Sodium Hydroxide	10 mg in 2 ml	Slightly soluble
4	Methanol	10 mg in 2 ml	Slightly soluble
5	Ethanol	10 mg in 8 ml	Slightly soluble
6	n – Butanol	10 mg in 7 ml	Slightly soluble
7	Chloroform	10 mg in 9 ml	Slightly soluble
8	DMF	10 mg in 7 ml	Slightly soluble
9	Acetone	10 mg in 4 ml	Slightly soluble
10	Dilute acetic acid	10 mg in MT 10ml	Insoluble
11	Ethyl acetate	10 mg in 13ml	Very slightly soluble
12	Petroleum ether	10 mg in MT 10 ml	Insoluble
13	Toluene	10 mg in MT10 ml	Insoluble
14	Carbon tetra chloride	10 mg in MT 10 ml	Insoluble
15	Acetonitrile	10 mg in MT 10 ml	Insoluble
16	Benzene	10 mg in MT 10 ml	Insoluble
17	Dichloromethane	10 mg in 9 ml	Slightly soluble
18	n-Hexane	10 mg in MT 10 ml	Insoluble
19	IPA	10 mg in 4 ml	Slightly soluble
20	Acetate buffer pH-3.5	10 mg in 1.5 ml	Slightly soluble
21	Acetate buffer pH-6.0	10 mg in 3ml	Slightly soluble
22	Phosphate buffer pH – 6.0	10 mg in 100ml	Insoluble
23	Phosphate buffer pH – 7.0	10 mg in MT 100ml	Insoluble
24	Phosphate buffer pH – 8.0	10 mg in 15 ml	Very slightly soluble

TABLE-3

MELTING POINT DETERMINATION

S.NO	DRUG NAME	METING POINT	AVERAGE VALUE	MELTING POINT RANGE
1.	METOPROLOL SUCCINATE	136^0C 136^0C 137^0C 136^0C 137^0C 136^0C	136.33^0C	$136\text{-}137^0$C
2.	TELMISARTAN	262^0C 262^0C 260^0C 262^0C 260^0C 262^0C	261.33^0C	$261\text{-}263^0$C

TABLE-4

OPTICAL CHARACTERISTICS OF METOPROLOL SUCCINATE BY (SIMULTANEOUS EQUATION METHOD)

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 275 nm	METOPROLOL SUCCINATE at 228.5 nm
1.	**Beer's law limits**	2-10 µg/ml	2-10 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	3973.065143	16396.05638
3.	**Slope (m)**	0.00606904	0.025073571
4.	**Intercept (c)**	0.000171429	0.000429365
5.	**Correlation-Co-efficient (r)**	0.999890784	0.999924063
6.	**Regression Equation (Y=mx+c)**	Y=0.00606904x +0.000171429	Y=0.025073571x +0.000429365
7.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.165511438	0.040313539
8.	**LOD**	0.19543612	0.143342449
9.	**LOQ**	0.59220668	0.434371057
10.	**Standard Error**	0.000364089	0.000087190

TABLE-5

OPTICAL CHARACTERISTICS OF TELMISARTAN BY (SIMULTANEOUS EQUATION METHOD)

S.NO	PARAMETERS	TELMISARTAN at 275 nm	TELMISARTAN at 228.5 nm
1.	**Beer's law limits**	2-10 µg/ml	2-10 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	27591.30003	53815.31603
3.	**Slope (m)**	0.035626349	0.140126825
4.	**Intercept (c)**	0.00174656	0.004501587
5.	**Correlation-Co-efficient (r)**	0.99993	0.99991
5.	**Regression Equation (Y=mx+c)**	Y=0.035626349x +0.00174656	Y=0.140126825 +0.004501587
6.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.02815996	0.009669374
7.	**LOD**	0.235200495	0.192697579
8.	**LOQ**	0.712728772	0.583932059
9.	**Standard Error**	0.002576656	0.008650838

TABLE- 6

QUANTIFICATION OF FORMULATION (METOSARTAN-25) BY SIMULTANEOUS EQUATION METHOD

Drug	Sample No.	Labeled amount ($mgtab^{-1}$)	Amount found ($mgtab^{-1}$)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1	25	25.34	101.38	100.76	1.2076	1.1986	0.033546
	2	25	25.54	102.18				
	3	25	25.12	100.48				
	4	25	24.67	98.68				
	5	25	25.11	100.44				
	6	25	25.34	101.38				
TEL	1	40	40.28	100.71	100.78	0.1649	0.01637	0.00458
	2	40	40.22	100.55				
	3	40	40.33	100.81				
	4	40	40.41	101.03				
	5	40	40.35	100.88				
	6	40	40.28	100.71				

* Mean of six observations

TABLE-7

INTRA DAY AND INTER DAY ANALYSIS OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY SIMULTANEOUS EQUATION METHOD

Drug	percentage	Amount present* ($\mu g\ mL^{-1}$)		Amount added ($\mu g\ mL^{-1}$)		Amount estimated* ($\mu g\ mL^{-1}$)		Amount recovered* ($\mu g\ mL^{-1}$)		% Recovery*		S.D.		% R.S.D.		S.E.	
		Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter
MET	80	2	2	3.2	3.2	5.25	5.24	3.25	3.24	101.56	101.25	0.890749	0.868696	0.885289	0.8663	0.098972	0.096522
	100	2	2	4	4	6.02	6.00	4.02	4.00	100.50	100.00						
	120	2	2	4.8	4.8	6.79	6.78	4.79	4.78	99.79	99.58						
TEL	80	3.2	3.2	5.12	5.12	8.37	8.37	5.17	5.17	100.98	100.98	0.301054	0.301054	0.27141	0.297141	0.03345	0.03345
	100	3.2	3.2	6.4	6.4	9.69	9.69	6.49	6.49	101.41	101.41						
	120	3.2	3.2	7.68	7.68	11.00	11.00	7.80	7.80	101.56	101.56						

* Mean of Three Observations

TABLE-8

RUGGEDNESS STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY SIMULTANEOUS EQUATION METHOD

Drug	percentage	Condition	% Recovery*	S.D	% R.S.D	S.E.
MET	80	Analyst 1	100.32	0.6100	0.6080	0.0381
		Analyst 2	101.98	0.1732	0.1698	0.0192
	100	Analyst 1	100.00	0.4330	0.4330	0.0481
		Analyst 2	101.25	1.0000	0.9876	0.1111
	120	Analyst 1	99.52	0.6361	0.6392	0.0706
		Analyst 2	100.00	0.3637	0.3637	0.04042
TEL	80	Analyst 1	101.11	0.1154	0.1142	0.01283
		Analyst 2	101.04	0.1097	0.1085	0.01218
	100	Analyst 1	100.95	1.1001	1.0897	0.1222
		Analyst 2	101.32	0.2020	0.6206	0.0224
	120	Analyst 1	101.41	0.1328	0.1309	0.0147
		Analyst 2	101.62	0.2821	0.2777	0.0313

*****Mean of six observations

TABLE -9

RECOVERY STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY SIMULTANEOUS EQUATION METHOD

Drug	percentage	Amount present* ($\mu g\ mL^{-1}$)	Amount added ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	Average (%) ± S.D.	% R.S.D.	S.E.
MET	80	2	3.2	5.21	3.21	100.22	99.91 ± 0.3579	0.3582	0.03977
	100	2	4	6	4	100.00			
	120	2	4.8	6.78	4.78	99.52			
TEL	80	3.2	5.12	8.38	5.18	101.05	101.28 ± 0.2419	0.2392	0.02688
	100	3.2	6.4	9.69	6.46	100.95			
	120	3.2	7.68	10.99	7.79	101.41			

* Mean of Three Observations

TABLE-10

OPTICAL CHARACTERISTICS OF METOPROLOL SUCCINATE BY (AREA UNDER CURVE METHOD)

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 227.5-214 nm	METOPROLOL SUCCINATE at 303-278.5nm
1.	**Beer's law limits**	2-10 µg/ml	2-10 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	329219.6297	35945.44762
3.	**Slope (m)**	0.502610238	0.055014762
4.	**Intercept (c)**	0.017690476	0.000487302
5.	**Correlation- Co-efficient (r)**	0.9999	0.9999
6.	**Regression Equation (Y=mx+c)**	Y=0.502610238x +0.017690476	Y=0.055014762x +0.000487302
7.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.002000426	0.01822363
8.	**LOD**	0.05869901	0.143102643
9.	**LOQ**	0.177875788	0.433102643
10.	**Standard Error**	0.02657721	0.00269465

TABLE-11

OPTICAL CHARACTERISTICS OF TELMISARTAN BY (AREA UNDER CURVE METHOD)

S.NO	PARAMETERS	TELMISARTAN at 227.5-214 nm	TELMISARTAN At 303-278.5 nm
1.	**Beer's law limits**	2-10 µg/ml	2-10 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	672549.3228	613867.9328
3.	**Slope (m)**	1.295958889	1.184860159
4.	**Intercept (c)**	0.109772222	0.080429365
5.	**Correlation-Co-efficient (r)**	0.999912089	0.999878024
5.	**Regression Equation (Y=mx+c)**	Y=1.295958889x +0.109772222	Y=1.184860159x +0.080429365
6.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.000774802	0.000847447
7.	**LOD**	0.148839775	0.213862987
8.	**LOQ**	0.451029621	0.648069658
9.	**Standard Error**	0.107476858	0.107247113

TABLE- 12

QUANTIFICATION OF FORMULATION (METOSARTAN-25) BY AREA UNDER CUVRE METHOD

Drug	Sample No.	Labeled amount ($mgtab^{-1}$)	Amount found ($mgtab^{-1}$)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1	25	25.17	100.68	99.71	0.68814	0.69013	0.019115
	2	25	25.10	100.43				
	3	25	24.79	99.16				
	4	25	24.79	99.16				
	5	25	24.79	99.16				
	6	25	24.92	99.68				
TEL	1	40	39.77	99.43	99.82	0.304543	0.305082	0.00846
	2	40	39.78	99.45				
	3	40	40.03	100.09				
	4	40	40.03	100.09				
	5	40	39.97	99.93				
	6	40	39.98	99.95				

***Mean of six observations**

TABLE-13

INTRA DAY AND INTER DAY ANALYSIS OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY AREA UNDER CUVRE METHOD

Drug	percentage	Amount present* (µg mL^{-1})		Amount added (µg mL^{-1})		Amount estimated* (µg mL^{-1})		Amount recovered* (µg mL^{-1})		% Recovery*		S.D.		% R.S.D.		S.E.	
		Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter
MET	80	2	2	3.2	3.2	5.21	5.21	3.21	3.21	100.31	100.31	1.4992	1.5053	1.4717	1.4801	0.166581	0.1667259
	100	2	2	4	4	6.08	6.06	4.08	4.06	102.00	101.50						
	120	2	2	4.8	4.8	6.96	6.99	4.96	4.99	103.30	103.30						
TEL	80	3.2	3.2	5.12	5.12	8.29	8.29	5.09	5.09	99.41	99.41	0.030000	0.030000	0.030187	0.030187	0.003333	0.003333
	100	3.2	3.2	6.4	6.4	9.56	9.56	6.36	6.36	99.38	99.38						
	120	3.2	3.2	7.68	7.68	10.83	10.83	7.63	7.63	99.35	99.359						

* Mean of Three Observations

TABLE-14

RUGGEDNESS STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY AREA UNDER CUVRE METHOD

Drug	percentage	Condition	% Recovery*	S.D	% R.S.D	S.E.
MET	80	Analyst 1	100.10	0.3579	0.3575	0.0397
		Analyst 2	100.31	0.4033	0.4027	0.0375
	100	Analyst 1	100.92	1.4648	1.4515	0.1627
		Analyst 2	101.25	0.4330	0.4276	0.0481
	120	Analyst 1	102.99	0.8653	0.8402	0.0961
		Analyst 2	99.79	0.2032	0.2019	0.0252
TEL	80	Analyst 1	99.39	0.0346	0.0348	0.0038
		Analyst 2	99.41	0.6051	0.6038	0.0593
	100	Analyst 1	98.97	0.4592	0.4640	0.0510
		Analyst 2	99.43	0.0866	0.0870	0.0096
	120	Analyst 1	99.52	0.1985	0.1995	0.0220
		Analyst 2	100.52	0.7032	0.7029	0.0221

*Mean of six observations

TABLE -15

RECOVERY STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY AREA UNDER CURVE METHOD

Drug	percentage	Amount present* ($\mu g\ mL^{-1}$)	Amount added ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	Average (%) ± S.D.	% R.S.D.	S.E.
MET	80	2	3.2	5.20	3.20	100.10	101.34 ± 1.4893	1.4697	0.1654
	100	2	4	6.04	4.04	100.92			
	120	2	6.4	6.97	4.97	102.99			
TEL	80	3.2	5.12	8.29	5.09	99.39	99.33 ± 0.2205	0.2220	0.0245
	100	3.2	6.4	9.54	6.34	99.09			
	120	3.2	7.68	10.84	7.64	99.52			

* Mean of Three Observations

TABLE-16

OPTICACHARACTERISTICS OF METOPROLOL SUCCINATE AND TELMISARTAN BY (FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 269 nm	TELMISARTAN at 243 nm
1.	**Beer's law limits**	10-50 µg/ml	16-80 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	130.0937143	1166.475676
3.	**Slope (m)**	0.00019381	0.002168349
4.	**Intercept (c)**	0.0000547619	0.000984127
5.	**Correlation-Co-efficient (r)**	0.9998	0.9999
5.	**Regression Equation (Y=mx+c)**	Y=0.00019381x +0.0000547619	Y=0.002168349x +0.000984127
6.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	5.161626985	0.461561591
7.	**LOD**	0.099303638	0.303226499
8.	**LOQ**	0.30092116	0.918868178
9.	**Standard Error**	0.00000556425	0.000897122

TABLE- 17

QUANTIFICATION OF FORMULATION (METOSARTAN-25) BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

Drug	Sample No.	Labeled amount ($mgtab^{-1}$)	Amount found ($mgtab^{-1}$)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1	25	25.47	101.88	101.01	1.34263	1.32916	0.037295
	2	25	25.47	101.88				
	3	25	24.82	99.28				
	4	25	25.47	101.88				
	5	25	25.17	101.88				
	6	25	24.87	99.28				
TEL	1	40	40.79	101.99	101.19	1.06046	1.04790	0.029457
	2	40	40.74	101.85				
	3	40	40.45	101.13				
	4	40	39.65	99.12				
	5	40	40.62	101.55				
	6	40	40.62	101.55				

* Mean of six observations

TABLE-18

INTRA DAY AND INTER DAY ANALYSIS OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

Drug	percentage	Amount present* ($\mu g\ mL^{-1}$)		Amount added ($\mu g\ mL^{-1}$)		Amount estimated* ($\mu g\ mL^{-1}$)		Amount recovered* ($\mu g\ mL^{-1}$)		% Recovery*		S.D.		% R.S.D.		S.E.	
		Intra	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**
MET	80	10	10	16	16	26.23	26.07	16.23	16.07	101.44	100.44						
	100	10	10	20	20	30.19	30.02	20.02	20.02	100.95	100.01	0.407962	0.317648	0.403897	0.316508	0.045329	0.035294
	120	10	10	24	24	34.15	34.15	24.15	24.15	100.63	100.63						
TEL	80	16	16	25.6	25.6	41.61	41.62	25.61	25.62	100.98	100.08						
	100	16	16	32	32	48.2	48.06	32.20	32.06	101.41	100.19	0.320052	0.26839	0.318756	0.267623	0.035561	0.029821
	120	16	16	38.4	38.4	54.61	54.63	38.61	38.63	101.56	100.59						

* Mean of Three Observations

TABLE-19

RUGGEDNESS STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

Drug	percentage	Condition	% Recovery*	S.D	% R.S.D	S.E.
MET	80	Analyst 1	101.46	1.7666	1.7412	0.1963
		Analyst 2	100.44	1.3024	1.3019	0.1534
	100	Analyst 1	100.10	1.4722	1.4707	0.1635
		Analyst 2	100.10	1.4722	1.4707	0.1635
	120	Analyst 1	100.62	1.2239	1.2164	0.1359
		Analyst 2	100.62	1.2239	1.2164	0.1359
TEL	80	Analyst 1	99.96	0.4355	0.4357	0.0483
		Analyst 2	100.02	0.1756	0.1755	0.0195
	100	Analyst 1	100.85	1.4923	1.4797	0.1658
		Analyst 2	100.18	0.3837	0.3829	0.0426
	120	Analyst 1	100.47	0.2594	0.2582	0.0288
		Analyst 2	100.55	0.1300	0.1293	0.0144

*****Mean of six observations

TABLE -20

RECOVERY STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

Drug	percentage	Amount present* ($\mu g\ mL^{-1}$)	Amount added ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	Average (%) ± S.D.	% R.S.D.	S.E.
MET	80	10	16	26.23	16.23	101.46	100.72 ± 0.6862	0.6813	0.07625
	100	10	20	30.02	20.02	100.10			
	120	10	24	34.15	24.15	100.62			
TEL	80	16	25.6	41.59	25.59	99.96	100.42 ± 0.4465	0.4446	0.04962
	100	16	32	48.27	32.27	100.85			
	120	16	38.4	54.58	38.58	100.47			

* Mean of Three Observations

TABLE-21

OPTICAL CHARACTERISTICS OF METOPROLOL SUCCINATE BY (GEOMETRIC CORRECTION METHOD)

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 217,225,232 nm	METOPROLOL SUCCINATE at 267,275,283 nm
1.	**Beer's law limits**	2-10 µg/ml	2-10 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	4328290	7038444
3.	**Slope (m)**	6.595441	-10.9788
4.	**Intercept (c)**	0.352474	15.74788
5.	**Correlation-Co-efficient (r)**	0.992645	0.9918
6.	**Regression Equation (Y=mx+c)**	Y=6.595441x + 0.352474	Y=10.9788x +15.74788
7.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.000154	-0.000095
8.	**LOD**	0.174649	0.59185
9.	**LOQ**	0.52924	1.79349
10.	**Standard Error**	0.065919	0.0435

TABLE-22

OPTICAL CHARACTERISTICS OF TELMISARTAN BY (GEOMETRIC CORRECTION METHOD)

S.NO	PARAMETERS	TELMISARTAN at 217,225,232 nm	TELMISARTAN at 267,275,283 nm
1.	**Beer's law limits**	3.2-16 µg/ml	3.2-16 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	2684981	8809124
3.	**Slope (m)**	5.23179	17.06563
4.	**Intercept (c)**	4.148641	-1.96577
5.	**Correlation-Co-efficient (r)**	0.99479	0.993169
6.	**Regression Equation (Y=mx+c)**	Y=5.23179x +4.148641	Y=17.06563x +1.96577
7.	**Sandell's sensitivity (µg/cm²/0.001 A.U)**	0.00019	0.0000608
8.	**LOD**	0.08945	0.102019
9.	**LOQ**	0.27105	0.30915
10.	**Standard Error**	0.068482	0.025638

TABLE- 23

QUANTIFICATION OF FORMULATION (METOSARTAN-25) BY GEOMETRIC CORRECTION METHOD

Drug	Sample No.	Labeled amount ($mgtab^{-1}$)	Amount found ($mgtab^{-1}$)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1	25	25.04	100.16	100.00	0.3019	0.3018	0.0083
	2	25	24.92	99.68				
	3	25	24.98	99.92				
	4	25	24.92	99.68				
	5	25	25.11	100.44				
	6	25	25.04	100.16				
TEL	1	40	40.28	100.70	100.17	0.3058	0.3052	0.0084
	2	40	40.09	100.23				
	3	40	40.03	100.07				
	4	40	40.03	100.07				
	5	40	39.91	99.77				
	6	40	40.09	100.23				

***Mean of six observations**

TABLE -24

RECOVERY STUDIES OF 50 % PREANALYZED FORMULATION (METOSARTAN-25) BY GEOMETRIC CORRECTION METHOD

Drug	percentage	Amount present* (μg mL^{-1})	Amount added (μg mL^{-1})	Amount estimated* (μg mL^{-1})	Amount recovered* (μg mL^{-1})	% Recovery*	Average (%) ± S.D.	% R.S.D.	S.E.
MET	80	2	3.2	5.20	3.20	100.11	99.88 ± 0.5521	0.5528	0.0613
	100	2	4	5.97	3.97	99.25			
	120	2	6.4	6.81	4.81	100.28			
TEL	80	3.2	5.12	8.34	5.14	100.39	100.14 ± 0.2145	0.2142	0.0238
	100	3.2	6.4	9.60	6.40	100.04			
	120	3.2	7.68	10.88	7.68	100.00			

*Mean of three observations

TABLE -25

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP – HPLC METHOD

S.NO	PARAMETERS (specifications as per ICH guidelines)	METOPROLOL SUCCINATE	TELMISARTAN
1.	Tailing factor (≤ 2)	1.5	1.25
2.	Asymmetrical factor (≤ 2)	2	1.33
3.	Capacity factor (> 2.0)	0.52	3.47
4.	Theoretical plate per unit length	50.99	197.08
5.	Theoretical plates (>2000)	764.86	2956.19
6.	Resolution (> 2)	7.05	

TABLE-26

OPTICAL CHARECTERSTICS OF METOPROLOL SUCCINATE AND TELMISARTAN (RP-HPLC METHOD)

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 230 nm	TELMISARTAN at 230 nm
1.	**Beer's law limits**	30-70 µg/ml	48-112 µg/ml
2.	**Molar absorptivity (L/mol/cm)**	6985886.506	40477589.52
3.	**Slope (m)**	11.80776378	87.32922264
4.	**Intercept (c)**	11.0639369	86.70867568
5.	**Correlation-Co-efficient (r)**	0.9990	0.9994
5.	**Regression Equation (Y=mx+c)**	Y=11.80776378x +11.0639369	Y=87.32922264x +86.70867568
6.	**LOD**	0.006488491	0.001988734
7.	**LOQ**	0.019662102	0.006026466
8.	**Standard Error**	14.60533931	128.6548409

TABLE-27

ASSAY OF FORMULATION (METOSARTAN-25) BY RP – HPLC METHOD

Drug	Sample No.	Labeled amount (mgtab^{-1})	Amount found (mgtab^{-1})*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1 2 3 4 5 6	25 mg	24.93 25.17 25.12 25.02 24.88 24.91	99.72 100.68 100.48 100.09 99.52 99.66	100.02	0.4736	0.4734	0.0132
TEL	1 2 3 4 5 6	40 mg	39.58 39.75 39.62 39.84 39.69 39.91	98.94 99.37 99.04 99.59 99.23 99.77	99.33	0.3189	0.3211	0.0089

*****Mean of six observations

TABLE -28

RECOVERY STUDIES OF FOEMULATION BY RP – HPLC METHOD

Drug	Percentage	Amount present (μg/ml)	Amount added (μg/ml)	Amount estimated (μg/ml)	Amount recovered (μg/ml)	% Recovery	Average (%) ± S.D	% R.S.D	S.E
MET	80	30	25	54.85	25.48	101.92	102.47 ± 0.9154	0.8933	0.1017
	100	30	30	60.54	31.06	103.53			
	120	30	35	64.84	35.69	101.97			
TEL	80	48	38.4	85.99	37.99	98.93	99.42 ± 0.5729	0.5763	0.0636
	100	48	48	96.24	48.24	100.05			
	120	48	57.6	105.19	57.19	99.28			

*****Mean of three observations

TABLE -29

OPTICAL CHARACTERISTICS OF METOPROLOL SUCCINATE AND TELMISARTAN BY HPTLC METHOD

S.NO	PARAMETERS	METOPROLOL SUCCINATE at 233nm	TELMISARTAN at 233nm
1	**Beer's law limit (μg/ml)**	1-5	1.6 – 8
2	**Correlation Coefficient (r)**	0.9995	0.9998
3	**Regression Equation (y=mx+c)**	y =469.3 x+338	y =1155x+ 1.706
4	**Slope (m)**	469.3	1155
5	**Intercept (c)**	338	1.706
6	**Standard Deviation**	2.10	0.75

TABLE-30

ASSAY OF FORMULATION (METOSARTAN-25) BY HPTLC METHOD

Drug	Sample No.	Labeled amount ($mgtab^{-1}$)	Amount found ($mgtab^{-1}$)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
MET	1	25 mg	24.82	99.28	99.62	0.5549	0.5570	0.0616
	2		24.89	99.59				
	3		24,85	99.39				
	4		25.05	100.21				
	5		25.09	100.34				
	6		24.73	98.91				
TEL	1	40 mg	40.12	100.31	100.35	0.9224	0.9191	0.1024
	2		40.22	100.57				
	3		40.02	100.05				
	4		39.88	99.72				
	5		40.82	102.05				
	6		39.77	99.44				

*****Mean of six observations

TABLE -31

RECOVERY STUDIES OF FOEMULATION (METOSARTAN-25) BY HPTLC METHOD

Drug	Sample. No	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average (%) ± S.D	% R.S.D	S.E
MET	1 2 3	1 1 1	1.6 2 2.4	2.61 3.07 3.41	1.61 2.07 2.41	100.62 103.50 100.41	101.51 ± 1.7265	1.7009	0.1918
TEL	1 2 3	1.6 1.6 1.6	2.56 3.2 3.84	3.58 4.22 4.85	2.58 3.22 3.85	100.78 100.62 100.26	100.55 ± 0.2663	0.2648	0.0295

*****Mean of three observations

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Anonymous. Elico User's Manual. Elico Private Limited, B-17 Sanathnagar, Industrial Estate Hyderabad, **1998**, 11.
2. Anonymous. European Directorate for the Quality of Medicines and Health care edQm.Revision-01, 1-3.
3. Anonymous. Shimadzu Instruction Manual AX-200 Digital balance. Shimadzu Corporation, Kyoto, Japan, **2001,** 42.
4. Anonymous. Shimadzu LC-10 ATVP High Performance Liquid Chromatography Instruction Manual. Shimadzu Corporation, Kyoto, Japan, **2001,** 11-2.
5. Anonymous. Shimadzu SPD-10 ATVP High Performance Liquid Chromatography Instruction Manual. Shimadzu Corporation, Kyoto, Japan, **2001,** 11-2.
6. Anonymous. Shimadzu Instruction Manual Pharamaspec UV-1700 series operation Guide. Shimadzu Corporation, Kyoto, Japan, **2001,** 6.2.
7. Anonymous. The British Pharmacopeia Volume- I&II. International edition, Office of British Pharmacopoeia Commission, London, **2009,** 3933-3937, 5872-5877.
8. Anonymous. The United States Pharmacopoeia Volume - II. United States of Pharmacopoeia Convention, USA, **2007**, 1263.
9. Anonymous. The Merck Index, 14th edition., Merck Research Laboratories, Merck & co., Inc,USA, **2006**, 1060, 1569.

10. Beckett A.H., and Stenlake J.B. Practical Pharmaceutical Chemistry. 4th edn, CBS Publishers and Distributors, New Delhi, **2007,** 278-300, 307-312.

11. Chatwal and Anand. Instrumental Methods of Chemical Analysis. Re-print., Himalayan Publishing House, Mumbai, **2007**, 1.2, 2.108, 2.168, 2.147.

12. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, and 27th October, **1994**, 1-5.

13. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 6th November, **1996**, 1-8.

14. Chandra Bose R.J., Sivanseyal G., Duraisamy K.K., Surender N. S., Ramasamy P.Validated RP-HPLC method for the simultaneous estimation of Ramipril and metoprolol tartrate in bulk and tablet dosage form, *Asian Journal of Biochemical and Pharmaceutical Research*, **2011**, 1(2), 171-177.

15. Douglas A. Skoog, Donald M. West., James E. Holler and Stanley Crouch. Fundamental of Analytical Chemistry. 8th edn, Thomson Asia Pvt. Ltd., Singapore, **2006**, 2.

16. Deshpande M. M., Mahajan M P., Sawant S. D. Simultaneous estimation of valsartan and hydrochlorothiazide in fixed dose combination in UV spectrometry, *International Journal of pharmaceutical sciences and Research*, **2012**, 3(1), 236-240

17. Francis Rouessac and Annic Rouessac. Chemical Analysis Modern Instrumental Methods and Techniques. English edn., John Willey and Sons, Ltd., New York, **2000**, 45-46.

18. Gary D. Christian. Analytical Chemistry. 6^{th} edn, John Willey and Sons Ltd., New York, **2005**, 11, 106, 107, 555.

19. Gupta S.C., and Kapoor V.K. Fundamentals of Mathematical Statistics. 9th edn., Sultan Chand and Sons, New Delhi, **1994**, 2.6, 3.20 - 3.28.

20. Gurdeep R.Chatwal and Anand. Instrumental Methods of Chemical Analysis. Re-print.Himalaya Publishing House, Mumbai, **2007**, 1.2.

21. http://www.google.co.in/#sclient=psy-b&hl=en&source=hp&q=telmisartan+pka+value

22. Ilango K., Shijimkumar P.S. Simultaneous estimation of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage form, *Asian Journal of Pharmaceutical and health sciences.* **2011,** 1(1), 12-15.

23. Kalyankar T.M., Mubeena Khan., Nalanda Rangari., Kakhe R.B. A rapid colorimetric method for the estimation of ammonia in Telmisartan in bulk and solid dosage form, *International Journal of pharma World Research*, **2010,** 1(2), 1-8.

24. Kumbhar S.T., Chougule G.K., Gajeli G.B., Tegeli V.S., Thoat Y.S. Shivsharan U.S. Visible spectrophotometric determination of Telmisartan from urine, *International of Journal of Pharmaceutical Sciences and Research*, **2011,** 2(5), 1254-1258.

25. Lakman V. Portale., Mrinalini C. Damle., Amol S. Khodke., Bothara K.G. A validated stability indicting HPTLC method for simultaneous estimation of Ramipril and Telmisartan, *International Journal of pharmaceutical Sciences Review and Research*, **2010,** 2(2), 35-39.

26. Lakshmi K.S., Lakshmi sivasubramanian., krishanu pal. Stability indicating HPTLC method for simultaneous estimation of Telmisartan and Ramipril in tablets, *International Journal of Pharmacy and pharmaceutical Sciences*, **2010,** 2(4), 127- 129.

27. Moreshwar N. Kulkarni., Rajeshwar V.Kshirsagar., Dinesh M.Sakarkar. Development and validation of spectrophotometric method for determination of Metoprolol succinate. *International Journal of Chem. Tech research,* **2009,** 1(4), 1273-1277.

28. Mitesh D Phale., Purnima D Hamrapurkar. A validated and simplified RP-HPLC of Metoprolol succinate from bulk drugs, *Asian jounal of Research Chem,* **2009,** 2(2), 119-122.

29. Phale mitesh D., Hamrapurkar., Purnima D. Optimization and Establishment of a validated stability-indicating HPLC method for study of the stress degradation behavior of Metoprolol succinate, *Journal of AOAC International,* **2010,** 93 (3), 911-916.

30. Patel Amit R., Chandrul Kaushal Kishor. Method development, validation and stability study for simultaneous estimation of Telmisartan and indapamide by RP-HPLC in pure and marked formulation, *International Journal on pharmaceutical and Biomedical Research*, **2011,** 2(1), 4-16.

31. Patel V.A., Patel P.G., Chaudhary B.G., Rajgor N.B., Rathi S.G. Development and validation of HPTLC method for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form, *International Journal of Pharmaceutical and Biological research,* **2011,** 1(1), 18- 24.

32. Popat B. Mohite., Ramdas B. pandhare., Vaidhum h. Bhaskar. Simultaneous estimation of Ramipril and Telmisartan in tablet dosage form by spectrophotometry, *Eursian Journal of Analytical Chemistry,* **2010,** 5(1), 89-94.

33. Patil U.P., Gandhi S.V., Sengar M.R., Rajmane V.S. A validated densitometric method for analysis of Telmisartan and Atorvastatin calcium in fixed dose combination, *Journal of the Chilean Chemical Society,* **2010,** 55(1), 94-96.

34. Raja Kumar Seshadri., Makarand Madhukar Desai., Thummala Veera Raghavaraju., Deepa Krishnan., Dama Venugopala Rao., Ivon Elisha Chakravarthy. Simultaneous Quantitative determination of Metoprolol, Atorvastatin and Ramipril in capsules by a validated stability-indicating RP-UPLC method, *science pharma,* **2010,** 78 (4), 821-834.

35. Rajendra Kakde., Nilesh Bawane. HPTLC method for simultaneous estimation of Metoprolol succinate and amlodipine besylate in pharmaceutical preparations, *Journal of Planar Chromatography – modern TLC*, **2009,** 22(2), 115-119.

36. Ramesh J., Kamala kanan D., Jambulingam M., Ravi Shankar M., Arul prakasam K.C. Development and validation of HPTLC method for the simultaneous estimation of Atorvastatin and Telmisartan in combined dosage form, *International Journal of Xenobiotics and Pharma Technology,* **2011,** 1(1), 9-23.

37. Rekha Gangola., Sunil Kaushik., Sharma. Spectrophotometric simultaneous determination of Hydrochlorothiazide and Telmisartan in combined dosage form. *Journal of Applied Pharmarceutical Science,* **2011,** 1(1), 46-49.

38. Rekha Gangola., Narndra Singh., Anand gaurav., Mukesh Maithani., Ranji Singh. Spectrophotometric simultaneous determination of Hydrochlorothiazide and Telmisartan in combined dosage form by dual wavelength method, *International Journal of Comprehensive Pharmacy,* **2011,** 2(4), 1-3.

39. Sagar B. Wankhede., Nitin R. Dixit and Sohan S. Chaitlange. Stability indicating HPTLC method for quantitative determination of Atorvastatin calcium and Metoprolol succinate in capsules, *Scholars Research Library,* **2011,** 3 (1), 1-7.

40. Singh Brijesh., Patel DK., Ghosh SK. Development of RP- HPLC method for simultaneous analysis of Metoprolol succinate and Hydrochlorothiazide in a tablet formulation, *Tropical Journal of pharmaceutical Reseach*, **2009,** 8(6), 539-543.

41. Senthamil selvan P., pal T.k. Chromatography-tandem mass spectrometry method for the simultaneous quantification of Metoprolol succinate and simuvastatin in human plasma, *Journal of Pharmaceutical and Biomedical Analysis*, **2008,** 49(3), 780-785.

42. Sathe S.R., Bari S., Surana S.J. Development of HPTLC method for the estimation of Metoprolol succinate in bulk and in tablet dosage form, *Indian Journal of Pharmaceutical Education & Research,* **2008,** 42(1), 32-35.

43. Sagar Tatane. Development of UV spectrophotometric method of Telmisartan in Tablet Formulation, *Journal of Advaces in pharmacy and Healthcare Research,* **2011,** 2(1), 23-26.

44. Santaji Nalwade., Vangala Ranga Reddy., Dantu Durga Rao., Inabathina Koteswara Rao. Rapid simultaneous determination of Telmisartan, Amlodipine besylate and Hydrochlorothiazide in combined poly pill dosage form by stability **–**

indicating UPLC, *Scientia Pharmaceutica*, **2011,** 79(1), 69-84.

45. Sethi P. D. Quantitative analysis of drug in Pharmaceutical formulations, 1st edn., CBS Publishers and Distributors, New Delhi, **2001**, 7-13.

46. Telekone R. S., Shah A. N., Khan, M.J. Deshpande S.V., and Mahaparale S. P., Spectrophotometric estimation of riluzole in tablets dosage form. *International Journal of Pharma. Research & Development –online*, **2010**, 2(6), 1-6.

47. Tripathi KD. Essentials of medical pharmacology. 6 th Edition, Jaypee Brothers' Medical Publishers Ltd. New Delhi, **2008**, 141, 489.

48. Vachhani kevin H., Patel Sathish A. Development of validation of first order derivative spectrophotometric method for simultaneous estimation of Metoprolol succinate and Olimesartan Medoxomil in tablets, *Journal of Pharmaceutical science and Bio Scientific Research*, **2011**, 1(2), 113-117.

49. Vijay kumar G., Murthy TEGK., Sambasiva Rao KBS. Validated RP-HPLC method for the esatimation of Telmisatan in serum samples, *International Journal of Reseach in pharmacy and Chemistry*, **2011,** 1(3), 701-704.

50. Vijayamirtharaj R., Rameah J., Jayalakshmi B., Hanas Bin hashim. Development and validation of RP-HPLC method for the simultaneous estimation of Telmisartan and Atorvastatin calcium in tablet dosage forms, *International Journal of Comprehensibe Pharmacy*, **2010,** 4(3), 1-4.

51. Wankhede S.B., Tajne M.R., Gupta K.R., Wadodkar S.G. RP-HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form**,** *Journal of pharmaceutical and Biomedical Analysis*, **2009,** 69(2), 298-302.

52. www.jpsbr.org/index-htm-files/18.pdf

53. www.ijbpr.com/admin/fckeditor/samples/phpl article/80-50-54.pdf

54. www.asap.unimelb.edu.au/soc/fac.

55. Www. en.wikipedia.org/wiki/Analytical_chemistry

56. www.sciencelab.com.

57. Yogesh Gupta. Isocratic RP-HPLC method development and validation for the simultaneous estimation of Ramipril and Telmisartan in tablet dosage form, *Asian journal of Pharmaceutical and Clinical Research*, **2009,** 2(4), 104-111.